# THE NUTRITION OF TURNIPS

BY R. M. WOODMAN

*Horticultural Research Station, Cambridge University*

(With Plate 1)

THE effects of varying the supply of certain nutrients on the turnip, Milan Strap-leaved, Purple Top, are described in this paper.

## EXPERIMENTAL

General methods have been described previously (Woodman, 1940). The author has found by experience that deficiency experiments with potash, phosphate, and boron, can be carried out satisfactorily only with glazed pots, as the wax on plant-pots boiled in paraffin wax is liable to become black, friable, and permeable to water; with nitrogen this does not matter, possibly because all nitrates are soluble, and hence even unwaxed plant-pots are satisfactory. The experiments with potash, phosphate, and boron, therefore, were conducted in glazed culture jars, holding about 46 lb. of the culture sand,

TABLE 1. *Concentrations of the important elements present in the nutrient solutions*

| Nutrient solution used | N p.p.m. | P p.p.m. | K p.p.m. | Ca p.p.m. | Mg p.p.m. | Fe p.p.m. | S p.p.m. | Na p.p.m. | B p.p.m. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Phosphorus experiment | | | | | |
| A | 49·44 | *87·36* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *210·84* | 0·0681 |
| B | 49·44 | *43·68* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *146·04* | 0·0681 |
| C | 49·44 | *21·84* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *113·64* | 0·0681 |
| D | 49·44 | *10·92* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *97·44* | 0·0681 |
| E | 49·44 | *5·46* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *89·34* | 0·0681 |
| F | 49·44 | *0·55* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *82·05* | 0·0681 |
| G | 49·44 | *Nil* | 44·88 | 18·06 | 10·10 | 1·01 | 32·30 | *81·24* | 0·0681 |
| | | | | Nitrogen experiment | | | | | |
| A | *65·92* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *120·45* | 0·0681 |
| B | *32·96* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *66·31* | 0·0681 |
| C | *16·48* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *39·23* | 0·0681 |
| D | *8·24* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *25·69* | 0·0681 |
| E | *4·12* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *18·92* | 0·0681 |
| F | *0·41* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *12·83* | 0·0681 |
| G | *Nil* | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | *12·15* | 0·0681 |
| | | | | Potassium experiment | | | | | |
| A | 32·96 | 8·19 | *44·88* | 13·55 | 2·53 | 1·01 | *22·31* | 66·31 | 0·0681 |
| B | 32·96 | 8·19 | *22·44* | 13·55 | 2·53 | 1·01 | *13·11* | 66·31 | 0·0681 |
| C | 32·96 | 8·19 | *11·22* | 13·55 | 2·53 | 1·01 | *8·51* | 66·31 | 0·0681 |
| D | 32·96 | 8·19 | *5·61* | 13·55 | 2·53 | 1·01 | *6·21* | 66·31 | 0·0681 |
| E | 32·96 | 8·19 | *2·81* | 13·55 | 2·53 | 1·01 | *5·06* | 66·31 | 0·0681 |
| F | 32·96 | 8·19 | *0·28* | 13·55 | 2·53 | 1·01 | *4·03* | 66·31 | 0·0681 |
| G | 32·96 | 8·19 | *Nil* | 13·55 | 2·53 | 1·01 | *3·91* | 66·31 | 0·0681 |
| | | | | Boron experiment | | | | | |
| A | 65·92 | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | 120·45 | *0·0681* |
| B | 65·92 | 8·19 | 22·44 | 13·55 | 2·53 | 1·01 | 13·11 | 120·45 | *Nil* |

All solutions contained these proportions of minor elements: 0·1847 p.p.m. of Mn as $MnSO_4$, $4H_2O$; 0·0682 p.p.m. of Zn as the sulphate; 0·0095 p.p.m. of Al as ammonium alum; 0·0164 p.p.m. of the ammonium radicle, $NH'_4$, partly as ammonium alum, and partly as ammonium sulphate; and 0·0153 p.p.m. of Cu as the sulphate. The elements combined with these in the above salts have been neglected in compiling the table.

while those with nitrogen were carried out in 9 in. waxed plant-pots, each of which held about 13 lb. of sand.

The concentrations of the important elements present in the nutrient solutions are given in Table 1 as p.p.m. The italicized figures for N, P, and K show the variations for these elements; the italicized figures for S and Na show the concomitant variations in these elements (K was supplied as the sulphate, and N and P as sodium nitrate and phosphate, respectively).

The experiments were conducted in the greenhouse as arrangements of randomized blocks. For the *phosphorus* experiment there were seven treatments (A–G, Table 1) replicated ten times, or seventy cultures. The temperature ranged from 50 to 80° F. The seed was sown 2 Aug. 1938, and germinated by 5 Aug. Singling was done on 12 Aug. and the harvest was 14 Nov. 1938. Each culture received 18·6 l. of its solution in lots of 400 c.c. three times weekly. With *nitrogen*, there were seven treatments (A–G) replicated sixteen times, making 112 cultures. The temperature varied from 50 to 80° F. The seed was sown 5 May 1939, and germination had occurred by 9 May; the seedlings were singled on 11 May, and the harvest was on 13 July 1939. Each culture received 10 l. of its solution in lots of 250 c.c. four times weekly. For the *potassium* experiment there were seven treatments (A–G) replicated ten times, a total of seventy cultures. The temperature was 50 to 80° F. Seed was sown 12 May 1939, and germination had occurred by 18 May. The seedlings were singled on 19 May, and the harvest was 13 July 1939. Each pot received 14·6 l. of its solution in lots of 400 c.c. about every 2 days. In the *boron* experiment, two treatments, A and B, were replicated ten times, making twenty cultures. The temperature varied from 50 to 80° F. The seed was sown 20 July 1939, and germinated by 26 July. The seedlings were singled to one per pot on 27 July, and the harvest was taken 12 Sept. 1939. Each pot received 12·6 l. of solution, in lots of 400 c.c. four times weekly.

## The numerical data obtained

The eleven values obtained for each culture have been previously discussed, and a full description of the tables of summaries of results, together with methods of interpretation, have been given (Woodman, 1940). The data for the present experiment are contained in such a table (Table 2). The roots of the turnips were taken to include, as in horticultural practice, the edible portion plus associated fibrous roots.

## Observations on the cultures and discussion of results

*Phosphorus experiment.* Within a fortnight, cultures with solution A had light green or yellowish leaves with marginal scorch. B gave similar plants with less marginal scorch, while C, D, and E gave darker plants with still less scorch. F and G gave dark green plants with red stalks; the cotyledons were dying. Seedlings with D and E were the largest.

In general, throughout the experiment, the four solutions with most phosphate, A, B, C, and D, yielded plants which were of variegated green, due to a tendency to chlorotic patches, the colour approaching nearer to the normal, uniform, dark green, as the phosphorus decreased. E gave leaves of normal green. F and G yielded small plants with normal green leaves surrounded by a paler margin. At harvest, the tops with A were etiolated, and the turnips were pale, with hardly a tinge of the purple characteristic of this turnip. Tops and bulbs improved as the concentration of phosphorus decreased, until an even, dark green sample of tops, and purple-topped bulbs, were obtained with E. F and G gave normal green tops with a pale edging to the leaves, the bulbs being small and purple-topped with F, but small and pale with G.

Weekly measurements of the average greatest breadth of the tops, and the average breadth at right angles to this, demonstrated that E always yielded the largest tops. At harvest, the measurements were, in cm.: A, 13·9 × 10·6; B, 22·0 × 18·5; C, 22·1 × 18·3; D, 31·1 × 26·6; E, 33·7 × 29·4; F, 16·8 × 13·7; and G, 10·8 × 8·2. The bulbs appeared to follow the same

order. Thus D and especially E gave much the largest plants, and some notion of the relative sizes of the cultures may be gained from Pl. 1, fig. 1. Up to the optimum treatment, with E, there was a large and continuous increase in size of bulbs and tops. With F, there was a great and sudden decrease to a small plant.

Table 2 bore out these observations: the fresh and dried tops with D and E were by far the largest, all other yields being small and statistically equal ($D = E > A = B = C = F = G$); for the fresh and dry roots and whole plants, the order was $E > D > A = B = C = F = G$. Thus E yielded by far the largest roots and whole plants, and D the next largest. The differences between the results for the different treatments for these six values were very strongly significant (SSS).

Too much available phosphate—and in these culture experiments all the phosphorus is available—evidently acts as a deterrent to the growth of this turnip, in contrast to lettuce (Woodman, 1940). The range of phosphorus most beneficial is quite short, and an inspection of Table 2 shows that it must lie between the limits D (10·92 p.p.m. of P) and F (0·55 p.p.m.).

Differences between results for both top/root ratios, and for the moisture contents of the tops, roots, and whole plants, were not significant (NS, Table 2).

*Nitrogen experiment.* A nitrogen experiment was first conducted alongside the phosphorus one, the amount of phosphorus present in the nitrogen solutions being 43·68 p.p.m., a concentration suitable to lettuce. Growth was poor, the largest turnips weighing 40–50 g. only. As good results were obtained in the phosphorus experiment with D and E, and poor ones with A, B, and C, it was apparent that 43·68 p.p.m. of P was too high. The nitrogen experiment was therefore repeated with a more suitable phosphorus content for the solutions (8·19 p.p.m., Table 1), and the results are tabulated in Table 2, nitrogen section.

On 2 June 1939, there was no apparent difference for the treatments except in size. In another week, there was a tendency to the development of a chlorotic reddish mixture in the older leaves with F and G. These leaves subsequently died, so that at harvest there was little difference in colour for different treatments. At harvest, the linear dimensions of the tops in cm. were: A, $38·5 \times 34·5$; B, $33·7 \times 29·7$; C, $29·1 \times 25·9$; D, $23·8 \times 19·0$; E, $19·5 \times 15·1$; F, $8·7 \times 5·5$; and G, $7·2 \times 3·8$. Throughout the whole of the experiment the sizes held these relative positions, so that the growth of tops might be said to be normal in the sense that an increase in size accompanied an increase in nitrogen. Pl. 1, fig. 2, shows specimen plants for the first three solutions. With all solutions the roots had a normal appearance.

Fresh and dried tops followed a similar order: $A > B > C > D = E > F = G$. The roots and whole plants, fresh and dried, also followed an identical order, $A > B > C > D > F = G$; $D = E$; $E = F = G$. Both orders demonstrated what might be called normal response, in that increasing amounts of available nitrogen caused increasing yields of dry and fresh matter of the roots, tops, and whole plants. Differences between these results for the seven treatments were all very strongly significant (SSS).

Differences for top/root ratios and moisture contents were also very strongly significant. The two ratios followed the same order, $G > F > A = B = C = D = E$, demonstrating insensitivity to nitrogen changes over the range 65·92–4·12 p.p.m., but a rapidly increasing proportion of top, relative to root, for quantities of nitrogen below 4·12 p.p.m. The moisture contents of tops, roots, and whole plants, were unaffected by changes in the concentration of nitrogen when high (thus, $A = B = C$ for all three values), but decreased with the lower concentrations of nitrogen.

*Potassium experiment.* With solutions F and G (Table 1) there was a strong tendency for the oldest leaves to become chlorotic, first at the margins, and then all over, and for this to be followed by withering or scorch and death. The process was fairly rapid, and affected leaves and petioles tended to become limp and lie flat. Later, cultures with solution E were affected similarly to some extent.

The full final symptoms of potash starvation could be summarized thus: A strong tendency for the leaf to become limp and lie flat, and for large portions to become dull

TABLE 2. *Summaries of results. Weights in g.*

| Description of data | | Treatment mean for A | B | C | D | E | F | G | Mean of all results | S.E |
|---|---|---|---|---|---|---|---|---|---|---|
| **Nitrogen experiment** | | | | | | | | | | |
| Tops, FW | SSS | 31·46 | 18·84 | 12·08 | 5·77 | 3·42 | 0·541 | 0·391 | 10·36 | 0·956 |
| | | A>B>C>D=E>F=G | | | | | | | | |
| Roots, FW | SSS | 146·17 | 99·73 | 55·39 | 26·99 | 12·28 | 0·519 | 0·183 | 48·75 | 6·68 |
| | | A>B>C>D>F=G; D=E; E=F=G | | | | | | | | |
| Whole plants, FW | SSS | 177·63 | 118·57 | 67·47 | 32·76 | 15·71 | 1·060 | 0·574 | 59·11 | 7·387 |
| | | A>B>C>D>F=G; D=E; E=F=G | | | | | | | | |
| Tops, DW | SSS | 2·553 | 1·654 | 1·122 | 0·592 | 0·383 | 0·081 | 0·053 | 0·919 | 0·085 |
| | | A>B>C>D=E>F=G | | | | | | | | |
| Roots, DW | SSS | 10·53 | 7·580 | 4·296 | 2·083 | 1·089 | 0·050 | 0·024 | 3·665 | 0·469 |
| | | A>B>C>D>F=G; D=E; E=F=G | | | | | | | | |
| Whole plants, DW | SSS | 13·08 | 9·234 | 5·418 | 2·675 | 1·472 | 0·131 | 0·077 | 4·584 | 0·527 |
| | | A>B>C>D>F=G; D=E; E=F=G | | | | | | | | |
| Top/root, FW | SSS | 0·230 | 0·202 | 0·228 | 0·224 | 0·311 | 1·982 | 4·698 | 1·125 | 0·340 |
| | | G>F>A=B=C=D=E | | | | | | | | |
| Top/root, DW | SSS | 0·257 | 0·229 | 0·265 | 0·293 | 0·364 | 2·844 | 3·761 | 1·145 | 0·304 |
| | | G>F>A=B=C=D=E | | | | | | | | |
| Tops, % moisture | SSS | 91·94 | 90·88 | 90·70 | 89·70 | 88·23 | 85·04 | 85·22 | 88·81 | 0·595 |
| | | A=B=C>E>F=G; A>D=E>F=G; B=C=D | | | | | | | | |
| Roots, % moisture | SSS | 92·78 | 92·04 | 92·09 | 92·13 | 90·26 | 89·24 | 81·43 | 89·99 | 1·058 |
| | | A=B=C=D=E>G; A>F>G; B=C=D=E=F | | | | | | | | |
| Whole plants, % moisture | SSS | 92·64 | 91·87 | 91·86 | 91·72 | 89·89 | 86·69 | 84·65 | 89·90 | 0·691 |
| | | A=B=C=D>F=G; A>E>F=G; B=C=D=E | | | | | | | | |
| **Phosphorus experiment** | | | | | | | | | | |
| Tops, FW | SSS | 3·065 | 8·285 | 8·564 | 22·51 | 25·70 | 3·399 | 1·209 | 10·39 | 2·729 |
| | | D=E>A=B=C=F=G | | | | | | | | |
| Roots, FW | SSS | 13·58 | 26·40 | 42·80 | 119·5 | 172·4 | 9·237 | 4·859 | 55·54 | 13·42 |
| | | E>D>A=B=C=F=G | | | | | | | | |
| Whole plants, FW | SSS | 16·64 | 34·68 | 51·36 | 142·0 | 198·1 | 12·64 | 6·068 | 65·93 | 15·73 |
| | | E>D>A=B=C=F=G | | | | | | | | |
| Tops, DW | SSS | 0·203 | 0·657 | 0·734 | 2·139 | 2·467 | 0·327 | 0·156 | 0·955 | 0·229 |
| | | D=E>A=B=C=F=G | | | | | | | | |
| Roots, DW | SSS | 0·689 | 1·729 | 2·453 | 7·450 | 10·54 | 0·658 | 0·411 | 3·419 | 0·826 |
| | | E>D>A=B=C=F=G | | | | | | | | |
| Whole plants, DW | SSS | 0·892 | 2·385 | 3·186 | 9·588 | 13·01 | 0·985 | 0·567 | 4·373 | 1·041 |
| | | E>D>A=B=C=F=G | | | | | | | | |
| Top/root, FW | NS | 0·612 | 0·344 | 0·226 | 0·374 | 0·157 | 0·633 | 0·291 | 0·377 | 0·127 |
| Top/root, DW | NS | 0·892 | 1·193 | 0·343 | 0·413 | 0·246 | 1·428 | 0·416 | 0·705 | 0·443 |
| Tops, % moisture | NS | 74·87 | 85·45 | 90·21 | 90·34 | 89·75 | 84·39 | 85·73 | 85·82 | 4·414 |
| Roots, % moisture | NS | 89·83 | 91·12 | 93·95 | 92·98 | 93·81 | 91·83 | 90·87 | 92·06 | 1·406 |
| Whole plants, % moisture | NS | 87·74 | 87·95 | 93·36 | 92·76 | 93·36 | 87·55 | 90·11 | 90·40 | 2·888 |

TABLE 2 (*cont.*)

| Description of data | | Treatment mean for A | B | C | D | E | F | G | Mean of all results | S.E. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Potassium experiment** | | | | | | | | | | |
| Tops, FW | SSS | 27·13 | 24·97 | 27·26 | 23·81 | 9·860 | 1·210 | 0·232 | 16·35 | 1·339 |
| | | | | | A=B=C=D>E>F=G | | | | | |
| Roots, FW | SSS | 143·19 | 131·46 | 129·06 | 105·29 | 34·58 | 1·820 | 0·233 | 77·95 | 5·808 |
| | | | | | A=B=C>D>E>F=G | | | | | |
| Whole plants, FW | SSS | 170·32 | 156·43 | 156·32 | 129·10 | 44·44 | 3·030 | 0·465 | 94·30 | 6·230 |
| | | | | | A=B=C>D>E>F=G | | | | | |
| Tops, DW | SSS | 2·588 | 2·413 | 2·456 | 1·948 | 0·680 | 0·100 | 0·028 | 1·459 | 0·1357 |
| | | | | | A=B=C>D>E>F=G | | | | | |
| Roots, DW | SSS | 10·25 | 9·154 | 8·486 | 6·368 | 1·723 | 0·097 | 0·020 | 5·156 | 0·4163 |
| | | | | | A=B>D>E>F=G; A>C; B=C>D | | | | | |
| Whole plants, DW | SSS | 12·84 | 11·57 | 10·94 | 8·316 | 2·403 | 0·196 | 0·048 | 6·616 | 0·5072 |
| | | | | | A=B>D>E>F=G; A>C; B=C>D | | | | | |
| Top/root, FW | SSS | 0·194 | 0·197 | 0·216 | 0·236 | 0·294 | 1·878 | 2·197 | 0·745 | 0·2887 |
| | | | | | F=G>A=B=C=D=E | | | | | |
| Top/root, DW | SSS | 0·258 | 0·267 | 0·289 | 0·311 | 0·399 | 1·821 | 1·438 | 0·683 | 0·1365 |
| | | | | | F=G>A=B=C=D=E | | | | | |
| Tops, % moisture | SSS | 90·20 | 90·23 | 91·09 | 91·84 | 93·01 | 91·29 | 82·57 | 90·03 | 1·328 |
| | | | | | A=B=C=D=E=F>G | | | | | |
| Roots, % moisture | SSS | 92·86 | 92·97 | 93·38 | 93·94 | 94·91 | 92·16 | 80·19 | 91·49 | 1·356 |
| | | | | | A=B=C=D=E=F>G | | | | | |
| Whole plants, % moisture | SSS | 92·48 | 92·58 | 92·97 | 93·58 | 94·51 | 92·17 | 83·89 | 91·74 | 1·053 |
| | | | | | A=B=C=D=E=F>G | | | | | |
| **Boron experiment** | | | | | | | | | | |
| Tops, FW | SSS | 38·78 | 2·012 | — | — | — | — | — | 20·40 | 1·997 |
| | | | | | | A>B | | | | |
| Roots, FW | SSS | 50·99 | 2·314 | — | — | — | — | — | 26·65 | 4·928 |
| | | | | | | A>B | | | | |
| Whole plants, FW | SSS | 89·77 | 4·326 | — | — | — | — | — | 47·05 | 6·485 |
| | | | | | | A>B | | | | |
| Tops, DW | SSS | 2·696 | 0·293 | — | — | — | — | — | 1·495 | 0·2057 |
| | | | | | | A>B | | | | |
| Roots, DW | SSS | 3·480 | 0·157 | — | — | — | — | — | 1·819 | 0·2912 |
| | | | | | | A>B | | | | |
| Whole plants, DW | SSS | 6·176 | 0·451 | — | — | — | — | — | 3·313 | 0·4739 |
| | | | | | | A>B | | | | |
| Top/root, FW | NS | 0·936 | 1·438 | — | — | — | — | — | 1·187 | 0·3267 |
| Top/root, DW | NS | 0·857 | 3·472 | — | — | — | — | — | 2·165 | 0·8414 |
| Tops, % moisture | SSS | 93·12 | 78·57 | — | — | — | — | — | 85·85 | 2·103 |
| | | | | | | A>B | | | | |
| Roots, % moisture | NS | 92·99 | 91·43 | — | — | — | — | — | 92·21 | 0·5775 |
| Whole plants, % moisture | SS | 93·16 | 85·28 | — | — | — | — | — | 89·22 | 1·490 |
| | | | | | | A>B | | | | |

grey-green; there may also be pale brown scorch round the top margin of the leaf, but usually the leaf drops off, especially when handled, sometimes even when its colour is practically normal.

The roots were normal in appearance at harvest. The tops then had the following linear dimensions in cm.: A, 37·1 × 33·3; B, 36·6 × 34·0; C, 37·0 × 33·2; D, 32·2 × 28·9; E, 30·3 × 25·3; F, 13·1 × 7·4; and G, 6·9 × 3·3. A strong degree of insensitivity to changes in concentration of potassium between the limits A and C was thus apparent.

Differences between results for the eleven values with the seven treatments were all very strongly significant (SSS, Table 2). Increase of available potassium beyond certain limits did not affect yield, especially of fresh matter. Thus A = B = C for the fresh tops, roots, and whole plants, and, in all six values for fresh and dry yields, A = B at least. The two top/root ratios were greatly increased—that is, the growth of tops, relative to roots, was greatly favoured—by lack or shortage of available potassium, though insensitive to increments above a certain amount (F = G > A = B = C = D = E). The moisture contents for tops, roots, and whole plants, were insensitive to alteration of available potassium except for absence of it (A = B = C = D = E = F > G).

*Boron experiment.* Two treatments were used: solution A, the best from the nitrogen experiment, and B, which was A without the 0·0681 p.p.m. of boron as borax (Table 1).

By 18 Aug. 1939, the only difference was that cultures without boron were smaller. By 25 Aug. serious marginal scorch of the outer leaves of cultures without boron had developed; the inner leaves were few in number, relatively long, narrow, and twisting. On 8 Sept., three without boron were dead and had to be harvested, and three were dying; all the old leaves of surviving plants had died and withered, and the new leaves were slightly darker, and relatively long and narrow, with some tendency to twist along the main axis and to furl over at the margins. The furled-over portions developed scorch and withered, so that the new leaves gradually wasted away to leave only the thicker lower portions of the midrib (Pl. 1, fig. 3), and then the plant died.

The harvest was taken before the turnips with A were fully grown, as otherwise those receiving B would all have been dead. The turnips were normal with A, but were small and rough-sided with B, with a tendency to rot and to lack swelling ability.

Except for the two top/root ratios and the percentage moisture content of the roots, where the results were not significant (NS), the other values were very strongly or strongly significant (SSS or SS, Table 2). For each of these eight values, A > B. The six yields with A for fresh and dry tops, roots, and whole plants, were all much larger than the yields for B, where boron was omitted.

## Summary

The influence of variations in the supply of some inorganic nutrients on the growth of the turnip in sand was investigated. Deficiency symptoms were noted, and a statistical comparison of yields was made.

Progressive diminution of available nitrogen led to a progressive diminution in yield. Similar diminution in available phosphorus led at first, in strong contrast to lettuce, to a continuous rise in yield and a less chlorotic plant, then at a relatively low concentration of phosphorus to an optimum yield, and finally to a rapid decrease in yield. Potassium deficiency gave a characteristic grey-green scorch and limpness of the foliage, together with leaf drop. The yield was not decreased greatly by a reduction of available potassium until a fairly low level was reached. Absence of boron resulted in a characteristic wastage of the foliage, and ultimately in the death of the plant; the turnips were tiny, rough-sided, and had a tendency to rot. The presence of 0·0681 p.p.m. of boron, as borax, yielded plants of normal size, health, and appearance.

I thank Mr T. W. McKean and Mr J. N. Leonard for their help in these experiments.

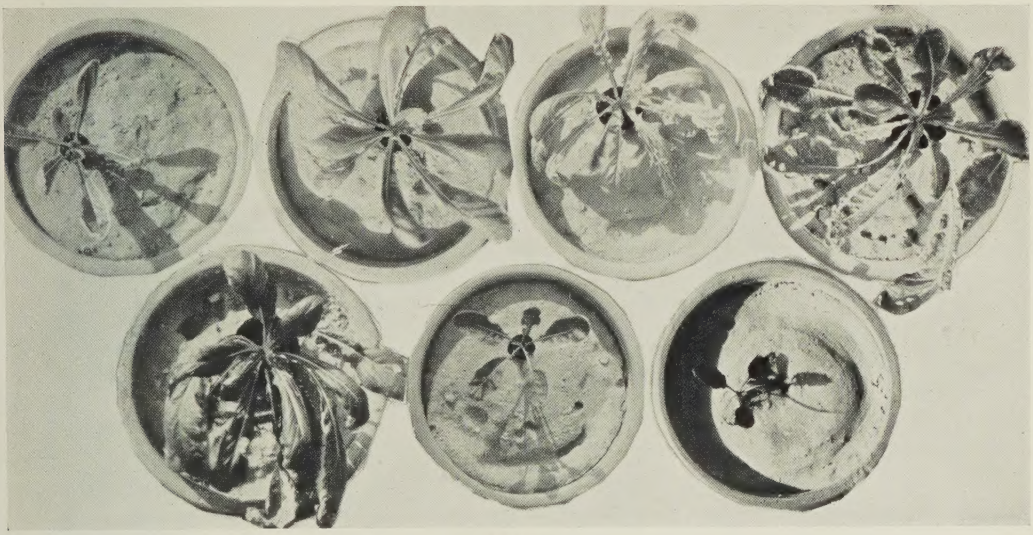


Fig. 1.


Fig. 2.

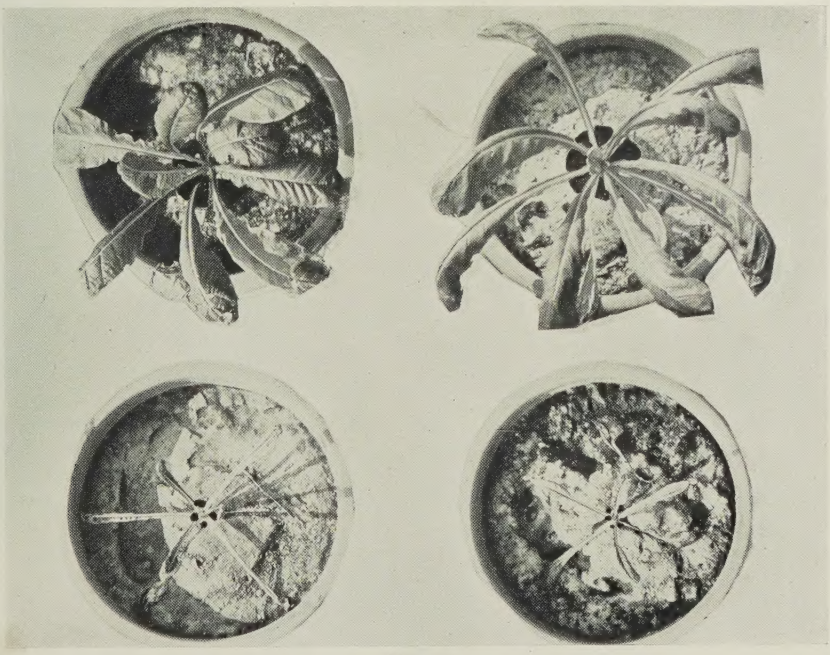
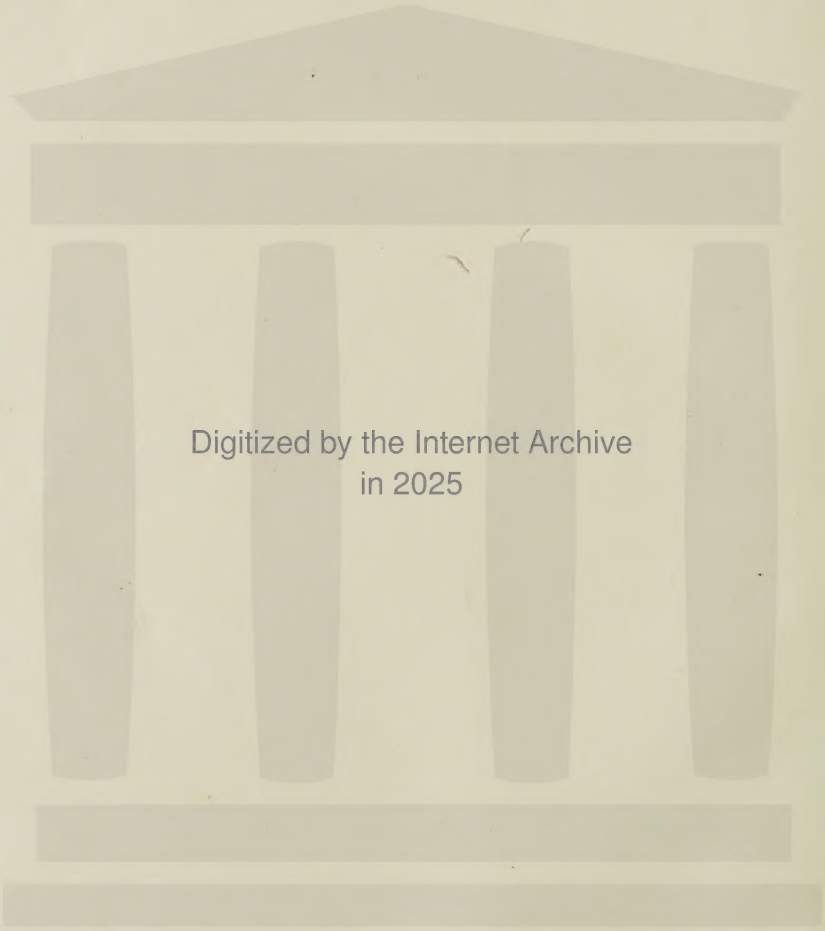


Fig. 3.

WOODMAN.—The Nutrition of Turnips (pp. 1–7)



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## EXPLANATION OF PLATE 1

Fig. 1. Cultures of turnips, with varying amounts of phosphorus. Top row, left to right: specimen cultures with solutions A, B, C, and D. Bottom row: with E, F, and G. The phosphorus was progressively reduced in passing from A to G (Table 1). The bulbs were blackened before reproduction. The culture jars have an internal diameter of about 10 in. Photo. 15 Oct. 1938.

Fig. 2. Specimen cultures, with solutions A, B, and C, nitrogen experiment. The labels are 6 × 4 in. Photo. 13 July 1939.

Fig. 3. The same culture solution was used for all four cultures except for the omission of boron for the two lower ones. The bulbs were blackened previous to reproduction. The inner diameter of the culture jars is about 10 in. Photo. 5 Sept. 1939.

(*Received* 4 *October* 1940)

# THE ULSTER METHOD FOR THE EXAMINATION OF FLAX SEED FOR THE PRESENCE OF SEED-BORNE PARASITES

By A. E. MUSKETT and J. P. MALONE

*Plant Disease Division, Ministry of Agriculture, Northern Ireland*

(With Plate 2)

*Polyspora Lini* Laff., the cause of Stem Break and Browning, and *Colletotrichum Lini* (Westerd.) Toch., the cause of Seedling Blight in the flax crop, are seed-borne, and the main purpose of this paper is to record the results of an attempt made to devise a rapid and accurate method for determining the percentage infection of flax seed samples with these parasites.

## Methods of seed examination for *Polyspora Lini*

(1) *Examination of seeds for the presence of spores.*

Each seed was placed in a drop of water on a microscope slide and allowed to soak. The coat was then removed, scraped with a scalpel, and the surrounding water examined for spores of *Polyspora*. A magnification of × 100 was employed. This method proved satisfactory in that the spores could easily be recognized, but it was laborious and irksome as routine procedure. Heavily infected seeds presented little difficulty, but in cases where a seed had become contaminated with the fungus to a slight extent and where only one or two spores were present, the examination was tedious and more difficult.

(2) *Examination of seeds, after incubation, for the production of acervuli and the presence of spores.*

Seeds were placed on moistened filter papers in Petri dishes as recommended for the examination of seed oats for *Helminthosporium Avenae* (Muskett, 1938) and incubated at room temperature (14–16° C.). After 4 days each seed was examined using a wide field binocular microscope (magnification × 60) for the presence of acervuli. In doubtful cases the seed was examined further for the presence of spores as in method (1). It was thought that this method might prove to be as satisfactory as method (1) and less tedious, but such was not the case. The acervuli could not always be recognized with ease, and the labour involved in submitting each doubtful case to further examination outweighed any advantage it might possess.

(3) *The Ulster method.*

*Examination of gel-media, upon which seeds are plated out, for the growth of the fungus.* As methods (1) and (2) were not altogether satisfactory it was decided to determine whether the problem could be more easily approached by plating out the seeds on agar medium poured into Petri dishes, and examining the medium around each seed for the growth of the fungus. Ten seeds were spaced equidistantly in each dish which was then incubated at 22° C. After 5 days an examination was made for the growth of *P. Lini*. Colonies of the fungus were found to be easily identified and, after a little experience, diagnosis could be made readily and rapidly by eye inspection. Preliminary trials showed this method to be promising and practicable, and it was decided to make further experiments to determine how it could be best applied. A range of agar media including water agar, malt extract agar with varying concentrations of malt extract, glucose agar and prune agar (Rawlins, 1933) were used but none was found to be better than 2% malt extract agar. Neither was it found advantageous to use acidified media with any of these nutrients as a base.

One point which emerged was the necessity for the frequent sterilization of forceps used for the transference of each seed from the sample to the dish. The pressure of the points of the forceps upon the seed may be sufficient to cause the transfer of spores from the seed to the forceps. This was proved

by streaking agar media with the points of sterilized forceps which had been plunged into a sample of infected seeds, squeezed and withdrawn. Colonies of *Polyspora* grew out along the sides of the streaks. The difficulty was overcome by dipping the points of the forceps in alcohol and flaming them after they had been used for picking out each lot of ten seeds.

After considerable experience the following technique was devised and has been employed successfully on a large scale.

The seed sample to be examined is thoroughly mixed and, if desired, further sampled by the method used in the testing of seeds (Mercer, 1938) until a sample approaching the size required is obtained. The seeds to be used are then plated out on 2% malt extract agar poured into Petri dishes (9·0 cm. diam.), ten seeds being spaced equidistantly in each dish. The points of the forceps are sterilized by dipping in alcohol and flaming after the plating out of each lot of ten seeds. The dishes are then incubated at 22° C. and after 5 days the medium around each seed is examined by eye inspection for the growth of *P. Lini*. Only very occasionally is it necessary to resort to microscopic examination.

## The size of seed sample

In the first place a sample of 100 seeds from each bulk of seed was examined, and although this was found to give an approximate estimate of the degree of infection, it was soon realized that the examination of a larger sample would be necessary for accurate estimation. For example: the following results were obtained when ten samples, each of 100 seeds, were selected at random from two well-mixed bulk samples known to be infected to a moderate degree with *P. Lini*:

| Bulk sample no. | % infection with *P. Lini* determined for each of 10 samples of 100 seeds |
|---|---|
| 1 | 19, 17, 22, 16, 18, 16, 19, 15, 19, 25 |
| 2 | 19, 18, 12, 12, 9, 15, 12, 15, 13, 11 |

If these samples are grouped in series of fives, each group thereby representing 500 seeds, then the following results are obtained by selecting at random ten such groups of five from each of the two bulk samples:

| Bulk sample no. | % infection with *P. Lini* calculated for each of 10 samples of 500 seeds |
|---|---|
| 1 | 18·4, 18·8, 20·0, 18·4, 19·8, 18·6, 19·4, 18·4, 18·8, 17·9 |
| 2 | 14·0, 13·2, 13·2, 14·0, 13·0, 14·2, 13·2, 14·2, 13·0, 13·0 |

It is thus seen that when samples of 100 seeds were examined, variations of from 16 to 25% and 9 to 19% occurred. When the sample was increased to 500 seeds the variations were reduced to from 17·9 to 20·0% and 13·0 to 14·2%. Therefore, if a sample of 500 seeds from a well-mixed bulk be examined a degree of accuracy to within 2% is obtained.

Whereas an approximate estimation of the amount of infection with *P. Lini* in any well-mixed seed sample may be obtained by the examination of a sample of 100 seeds, it is necessary to examine a sample of the order of 500 seeds if accuracy is required.

## The spread of infection by the mixing of seed

The necessity for the thorough mixing of seed samples before drawing final samples for testing purposes is obvious, but when it was found that the spores of *P. Lini* could be transferred from seed to seed by the use of forceps, it was decided to determine to what extent infection was likely to be spread in a seed sample during the process of mixing. In

order to clear up this point, 1 cwt. of each of two bulk samples of seed known to be infected with *P. Lini* and which required mixing, were utilized. Each was emptied on a tarpaulin sheet spread on a hard floor and ten small samples were drawn at random before mixing. The heap was then thoroughly mixed for about 15 min. using wooden shovels and rakes. After mixing, ten further samples were drawn from each heap. One hundred seeds from each sample were examined, whereby results were obtained for the examination of 1000 seeds for each of the two bulk samples before and after mixing. In the case of sample 1 the percentages of infection with *P. Lini* obtained before and after mixing were 22·0 and 18·6 respectively, whereas for sample 2 they were 16·4 and 13·6. The results obtained were of the same order in each case, and it can therefore be assumed that infection is not spread with a seed sample during normal mixing operations.

### Comparison of Ulster and Danish methods

Coincident with this investigation in Northern Ireland, Stahl & Kjaer[1] reported on method being used in Denmark: "400 seeds of each sample were examined by means o lens and each suspicious seed was placed in a drop of a 0·1 % water solution of cotton bl and kept there for one hour. Then the seeds were scraped with a scalpel and removed fr the drop and placed under a cover glass. A microscopic examination (magnification ×1 revealed the presence or non-presence of spores of *Polyspora Lini*. Those seeds wh delivered spores to the liquid, were considered as infected, the others as free and on t basis of the 400 seeds examined the percentage of attack was calculated."

Except for two points this method corresponds closely with method 1 described in t paper, the differences being the selection of suspicious seeds for microscopic examinat and the use of cotton blue as a stain. The following results expressed in terms of percent infection were obtained when seven seed samples were examined by each method. Samp of 400 or 500 seeds were used in each case:

| | Sample no. | | | | | | |
|---|---|---|---|---|---|---|---|
| Method | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Ulster | 5·0 | 14·5 | 3·75 | 19·6 | 14·4 | 10·8 | 12·2 |
| Danish | 0·0 | 4·0 | 1·25 | 11·4 | 9·6* | 5·2 | 5·4† |

* In this case 143 seeds were selected as suspicious and on examination revealed infection to the extent of 9·6 % of the gross sample of 500. The remaining 357 seeds were then examined by the Ulster method by w a further 7·6 % gross infection was recorded. This brought the total for this sample to 17·2 %.

† Here 107 seeds were selected as suspicious and when examined gave a percentage infection of 5·4 % of the gross sample. The remaining 393 when examined by the Ulster method showed a further infection of 5· thus bringing the total to 10·6 %.

It would thus appear that the Ulster method is more suitable for the accurate examinat of flax seed samples for infection with *P. Lini*.

### Method of seed examination for *Colletotrichum Lini*

Colonies of *C. Lini* growing on malt extract agar are easily recognized by their salr pink colour, and it soon became evident that the Ulster method was suitable for the deter nation of the infection of flax seed samples with this parasite. Some 500 samples have b examined for *C. Lini* in addition to *P. Lini* during the early months of 1940, with satisfactory results.

Details of the Danish method were kindly provided by Stahl & Kjaer in the course of correspondence with the writers.

### Examination for other fungi and bacteria

From experience already gained it would seem that it may be possible to extend the use of this method to include other seed-borne parasites of flax should they prove to be readily recognized by their growth on media. Complications might ensue owing to antagonism whereby the growth of one organism would be suppressed by another, but so far no serious difficulty of this kind has been experienced with regard to the identification of *P. Lini* and *C. Lini*, although in samples heavily contaminated with moulds and bacteria a keener and more careful inspection is needed.

### Determination of the country of origin of seed

One interesting point which emerged during the course of the work is the possibility of the identification, by means of the fungus flora observed, of the country of origin of seed. For instance, it has been found possible to identify samples of Canadian seed by the frequent occurrence of a fairly easily recognized species of *Alternaria*. It is not suggested, nor has sufficient evidence been obtained to show, that such determination is infallible, but in several cases, seed samples, the country of origin of which at the time of examination was unknown, were determined as of Canadian origin owing to the frequent occurrence of this mould, and in every case the determination was afterwards found to be correct.

### Determination of the age of seed

Seed which is one year old or more tends to show less contamination with moulds and bacteria. It is not yet known to what extent seed-borne parasites, such as those under investigation, tend to die out with the passage of time, but it has already been noted that the degree of contamination with viable moulds and bacteria decreases as the storage life of the seed increases. When samples of old seed were examined they were found to be comparatively "clean", and the application of this test should prove useful in assisting to distinguish between new and old seed.

## Discussion

Of the three methods employed for the examination of flax seed samples for infection with the parasites *P. Lini* and *C. Lini*, that involving the plating out of the seeds on malt extract agar and examining the medium for the growth of the fungi concerned has proved the most successful and satisfactory. Although its application requires more apparatus and equipment than may be necessary for other methods, nothing more is needed than will be found in a well-equipped laboratory. It is especially suitable for routine work where large numbers of samples need to be examined, as it involves little eye strain; after some practice and under supervision, it can be carried out by a well-qualified laboratory assistant.

The fact that the estimation is based upon observations on the living fungi means that only viable infection is recorded, and this is an additional advantage.

A comparison of the Danish and Ulster methods resulted consistently in the recording of a lower percentage infection with *P. Lini* when the Danish method was employed. This is due to the fact that the fungus can be carried by seeds not regarded as suspicious. Diseased flax seeds may often be detected by the naked eye through their dullness, dark colour, and general unhealthy appearance, an observation first made by Pethybridge & Lafferty (1918):

it is seed of this type which Stahl & Kjaer have termed suspicious. When the Danish method was being tested every seed which showed the slightest abnormality in appearance was classed as suspicious, and the fact that when the "healthy" remainder was examined by the Ulster method, a further considerable proportion of infected seeds was recorded, indicates clearly that the selection of suspicious seeds alone for further examination cannot be regarded as an entirely satisfactory procedure. It is also possible for seed-borne parasites to be present as mycelium in a non-sporing state in the outer layers of the seed coat. Infected seeds of this type would be discounted by any method which relies upon the presence of spores as a criterion for infection. The Ulster method overcomes this difficulty in that by plating out the seeds on a nutrient medium the mycelium is given the opportunity of growing out from the seed coat and producing a visible colony in the neighbourhood of the seed.

## Summary

1. An account is given of the investigation of likely methods for the examination of flax seed samples for the presence of such seed-borne parasites as *Polyspora Lini* and *Colletotrichum Lini*. An outline of the Ulster method which was adopted as being the most suitable is as follows:

The seed sample to be examined is thoroughly mixed and the seeds plated out on 2% malt extract agar poured into Petri dishes (9·0 cm. diam.); ten seeds are spaced equidistantly in each dish. If only a general opinion upon the health of the seed sample is required, 100 seeds are used, but if an accurate estimation of the degree of infection is required, 500 seeds are examined. Forceps are used for the transfer of each seed to the medium, and the points of these are sterilized by dipping in alcohol and flaming after the plating out of each lot of ten seeds. The dishes are then incubated at 22° C., and after 5 days the medium around each seed is examined for the growth of parasitic organisms.

2. Although the spores of *P. Lini* may be spread in a seed sample when forceps are used for the selection of seeds, the spread of the fungus does not occur during the normal operations employed in the handling and mixing of seed.

3. The use of the Ulster method has been compared with that used in Denmark.

4. Examples are cited where it has been possible by the use of this method to determine the country of origin of flax seed.

5. Indications have also been obtained which suggest its suitability in assisting towards the distinction between new and old seed.

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Fig. 1.

Fig. 2.

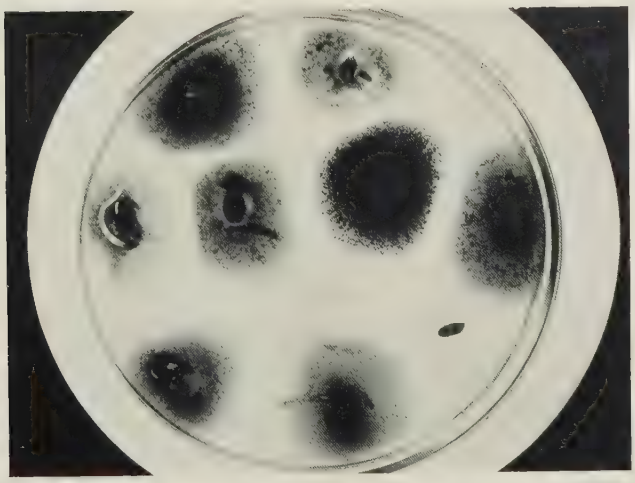

Fig. 3.

MUSKETT AND MALONE.—THE ULSTER METHOD FOR THE EXAMINATION OF FLAX SEED FOR THE PRESENCE OF SEED-BORNE PARASITES (pp. 8–13)

## EXPLANATION OF PLATE 2

Fig. 1. Three colonies of *Polyspora Lini* growing from infected flax seeds plated on malt extract agar. Note the typical striate growth shown by each colony. At first creamy white in colour, the colony darkens with age. Slightly reduced.

Fig. 2. Single colony of *P. Lini* growing from an infected flax seed. ×2.

Fig. 3. Eight colonies of *Colletotrichum Lini* growing from infected seeds plated on malt extract agar. The fungus is recognized by the bright salmon pink colour of the colony, its close matted growth near the surface of the medium and the rather scanty production of aerial mycelium. The occurrence of different strains of this fungus may lead to a slight variation in growth characteristics, but the differences observed have not prevented satisfactory diagnosis. Slightly reduced.

(*Received* 15 *October* 1940)

# SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

## VI. THE EFFECT OF PLANT NUTRITION UPON DISEASE RESISTANCE

By S. D. GARRETT

*Rothamsted Experimental Station, Harpenden, Herts*

Field observations on the effect of fertilizer treatment upon occurrence of take-all in wheat, found in the literature, are concerned chiefly with the beneficial influence of superphosphate in the control of this disease on Australian soils, some of which are notoriously deficient in phosphate (Griffiths, 1933; Samuel, 1934). Few precision experiments have been reported. Rosen & Elliott (1923) carried out an experiment involving three treatments, viz. farmyard manure at the rate of 10 tons/acre, a 4-8-3 general fertilizer at the rate of 400 lb./acre, and burnt lime at the rate of 1 ton/acre. The percentages of whiteheads on the different $\frac{3}{4}$ acre plots at harvest were as follows: control plot (untreated) 80%, farmyard manure plot 45%, artificial fertilizer plot 7% and lime plot 95%. The average yields per acre were as follows: untreated plot 4 bushels, manure plot 14·3 bushels, fertilizer plot 18·4 bushels, and lime plot a total failure. The action of lime in increasing the disease was elucidated by Garrett (1936), since when some striking examples of its disease-promoting effect have been noted on English farms. The remaining data of Rosen & Elliott indicate that nutrition of the host plant may be a factor of importance in determining the influence of infection upon crop yield. Whilst adequate manuring may not appreciably check the mycelial advance of *Ophiobolus* along the root system to the crown, it may yet minimize the influence of infection upon the plant's growth and yield. Doughty *et al.* (1929), in a survey of a field experiment upon the top dressing of wheat with $1\frac{1}{2}$ cwt. sulphate of ammonia, recorded a mean incidence of 25% of whiteheads in four top-dressed plots, as against a mean of 33% for the four untreated control plots.

The previous papers of this series (Garrett, 1936 et seq.) dealt with the effect of soil conditions upon the activity and survival of the causal fungus; the experiment here described is concerned with the effect of soil conditions upon the resistance to disease of the host plant.

### Experimental

Wheat plants grown in sand culture under five different manurial treatments were inoculated at a suitable stage with *Ophiobolus* and the development of disease recorded at the end of the experiment, together with its effect upon the yield of grain. The sand-culture technique was adapted from that devised by Gregory & Crowther (1928) for the first of a series of experiments upon the nutrition of the barley plant. Gregory & Crowther grew three barley plants in 30 lb. of sand per pot. In this experiment with *Ophiobolus*, single wheat plants were grown in 7 in. amber glass flower pots holding 10 lb. of washed sand; the amounts of nutrients given per pot were therefore one-third of those given by Gregory & Crowther to each of their pots, but the amounts per plant were the same. Where a pathogenic organism is concerned single plant pot culture has one important advantage over the more usual method of growing three or more plants in a pot; by the latter method, surviving plants in a pot derive a nutritional advantage if one or more of the original complement is killed or seriously retarded in growth by the disease.

Five nutrient treatments were compared, each nutrient series containing eighteen inoculated pots and six uninoculated or control pots. The treatments were as follows:

| | N | $P_2O_5$ | $K_2O$ |
|---|---|---|---|
| NPK | 0·50 | 0·17 | 0·33 |
| PK $\frac{1}{3}$N | 0·17 | 0·17 | 0·33 |
| NK $\frac{1}{3}$P | 0·50 | 0·057 | 0·33 |
| NP $\frac{1}{3}$K | 0·50 | 0·17 | 0·11 |
| $\frac{1}{3}$ (NPK) | 0·17 | 0·057 | 0·11 |

In the full nutrient or NPK series, each plant received the same amounts of N, P and K as were given by Gregory & Crowther to their barley plants. In three of the remaining series, each of the three nutrients was deficient in turn by two-thirds of the full amount. In the fifth series, all three nutrients were present in only one-third of the full amount. The composition of the full nutrient solution actually employed was similar in respect of all constituents except phosphate to one of those used by Richards & Shih (1940) and was as follows:

| | g. per pot |
|---|---|
| $NaNO_3$ | 1·52 |
| $Ca(NO_3)_2.4H_2O$ | 2·11 |
| $Na_2HPO_4.12H_2O$ | 0·833 |
| $CaCl_2.6H_2O$ | 0·123 |
| $MgSO_4.7H_2O$ | 0·417 |
| $K_2SO_4$ | 0·617 |

Subsequent to planting, an addition was made to each pot of 0·0033 g. $MnSO_4$ and of 0·037 c.c. of a saturated solution of $FeCl_3.6H_2O$; each of these was given in 100 c.c. of water.

In setting up the experiment, the complete solution for each manurial series was made up in amount sufficient for the twenty-four pots to be filled, but with omission of the sodium phosphate. The phosphate solution, which had previously been adjusted to *p*H 6·9, was added to the aliquot of nutrient solution measured out for each individual pot, and the resulting fine suspension shaken and poured onto the sand. After all the pots had been set up, three seeds of Red Marvel spring wheat were sown in each pot on 26 Mar., and the resulting seedlings thinned to one per pot one week later. The pots were randomized on four parallel benches in a large compartment of the glasshouse.

The question of inoculation received particular consideration. In the field, the degree of injury caused by the disease must largely depend upon the stage of plant growth at which the fungus reaches the crown of the plant. The importance of the spatial interval between inoculum and crown of the plant was demonstrated by Fellows & Ficke (1934) for one particular soil type. The time interval between contact of the root with the inoculum and infection of the crown by the fungus depends, however, not only upon the spatial interval between inoculum and crown, but also upon rate of growth of the fungus along the roots (Garrett, 1936). This relationship was well demonstrated by the experiments of Winter (1939), who also derived mathematical formulae expressing the relationship of disease injury to amount and distribution of inoculum in the soil, on the one hand, and to soil conditions and the resulting growth rate of the runner hyphae, on the other (Winter, 1940).

Although plants may be killed in the early stages of growth in the field, the disease is more commonly expressed in the development of whiteheads; production of the disease in this form was therefore made the object of this pot experiment. If the inoculation were to take effect too soon, the plants would be killed before heading; if too late, grain would develop in the ears before the disease had had time to cause serious interference. It was finally decided to bury a 0·25 cm. layer (50 g.) of sand + 3% cornmeal inoculum at a depth of 10 cm. below the seed and 5 cm. above the bottom of the pot; the seed was planted at a depth of 2 cm. Unfortunately, this inoculation was rendered completely ineffective through waterlogging. During the first 3 weeks of the experiment, the moisture content of the sand was maintained at 70% saturation, the drainage hole at the bottom of the glass pot having been plugged with paraffin wax. Insufficient allowance was made, however, for the sinking of the water in the sand, which proceeded to such an extent as to involve the inoculum layer in the saturated or subsaturated zone. For this reason, presumably, no infection occurred in any pot from the original inoculum layer, as was verified at washing out of the roots after harvest. On 1 May, approximately one month after planting, the seedlings were reinoculated by the insertion of two 1 in. lengths of infected wheat straw into the sand on either side of and immediately below the crown. This second inoculation proved to be effective and fortunately timed.

In the earlier stages of growth, but not later, the plants required support from a wire ring. The moisture content of the sand was maintained at 70% saturation for the first 3 weeks, but after that, the paraffin plugs were removed from the drainage holes, and the pots stood in glass saucers and watered from a can. Neither rust nor mildew developed on leaves or stem of any plant, nor did any mould growth subsequently appear upon the whiteheads. A severe infestation of aphides threatened to develop, but was checked by fumigation with nicotine on two consecutive occasions. A test exposure of growing Petri dish colonies of *Ophiobolus* to a nicotine fumigation had previously shown that the growth of the fungus suffered no perceptible check from the fumigant.

The ears emerged during the first week of June; towards the end of the month, whiteheads appeared in series $\frac{1}{3}$(NPK) and subsequently in other series. No precise count of whiteheads was possible, however, owing to the various gradations between completely empty whiteheads and well-filled heads. The ears of the plants were not harvested until the ear-chaff had completely lost every trace of green and the ears were judged to be almost dead ripe; the phosphate-deficient series, NK$\frac{1}{3}$P, were some days later in ripening, and were harvested a few days after the other series. The ears were placed in labelled paper bags; the grain was rubbed out of each ear and weighed one month later.

After harvesting the ears, the root systems of the plants were washed out for examination. At this time, the *p*H of the sand was tested in three pots of each series; values were just over seven in almost every pot, and the reaction of the sand would therefore have been favourable to the progress of infection in every series. Inspection of the root systems after washing out showed that satisfactory infection of the roots from the straw inoculum had occurred in every inoculated pot of the experiment. After washing out, the plants were split up into their constituent tillers, and classified according to the degree of visible infection of tiller-base and root system. The number of tillers with *Ophiobolus*-blackened stem-bases was recorded for each experimental series; doubtful cases in which blackening was just commencing were given a half mark, and the halves subsequently added up to the nearest whole number. At the same time, the number of tillers with majority of roots severely infected was also recorded for each series. In the remaining tillers the root systems were also extensively infected, but a majority of the roots could be classed as only moderately to lightly infected, with a proportion which were apparently healthy (see Table 1).

TABLE 1. *Degree of visible infection of stem-base and root system of ear-bearing tillers*

| | Mean no. of ear-bearing tillers per plant | % tillers with *Ophiobolus*-blackened stem-bases | % tillers with majority of roots severely infected |
|---|---|---|---|
| NPK | 5·5 | 20 | 66 |
| PK$\frac{1}{3}$N | 4·3 | 21 | 35 |
| NK$\frac{1}{3}$P | 4·9 | 41 | 68 |
| NP$\frac{1}{3}$K | 4·8 | 39 | 79 |
| $\frac{1}{3}$(NPK) | 4·3 | 52 | 65 |

In accordance with expectation, deficiency of nitrogen reduced tillering to a greater extent than deficiency of phosphate or of potash. The lowest percentage figures for *Ophiobolus*-blackened stem-bases are those of 20 and 21 for series NPK and PK$\frac{1}{3}$N, respectively. Series NK$\frac{1}{3}$P and NP$\frac{1}{3}$K are approximately equal with 41 and 39%, respectively, whilst series $\frac{1}{3}$(NPK) is highest with over 50% of blackened stem-bases. Infection figures for the root systems agree only in a general way with those for the stem-bases. Thus the percentage of tillers with majority of roots severely infected was of the same order (65–68%) for three series, viz. NPK, NK$\frac{1}{3}$P and $\frac{1}{3}$(NPK). It was much lower than this for series PK$\frac{1}{3}$N (35%) and somewhat higher for series NP$\frac{1}{3}$K (79%). Thus, whilst a deficiency of P or K, or of all three nutrients together, tends to increase the degree of visible infection, a deficiency of N appears to reduce it. The healthier appearance of the N-deficient root systems at the time of washing out was particularly striking.

Figures for mean grain weight per plant in each series may next be considered (Table 2).

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TABLE 2. *Mean grain weight per plant (g.)*

| | Control | Inoculated | % reduction in yield due to infection |
|---|---|---|---|
| NPK | 8·31 ± 0·37 | 8·07 ± 0·55 | 3 |
| PK⅓N | 7·88 ± 0·56 | 5·97 ± 0·37 | 24 |
| NK⅓P | 5·80 ± 0·37 | 2·93 ± 0·32 | 49 |
| NP⅓K | 8·22 ± 0·28 | 6·51 ± 0·45 | 21 |
| ⅓(NPK) | 6·83 ± 0·17 | 3·86 ± 0·40 | 43 |

The following conclusions concern the significance of the differences in Table 2.

(1) In the control pots, deficiencies of N and of K, respectively, failed significantly to depress the yield below that of the full nutrient series, NPK. Deficiency of P greatly reduced yield; deficiency of all three nutrients, however, resulted in a significantly smaller depression in yield.

(2) In the inoculated pots, a significant depression in yield occurred in all four deficiency series.

(3) Infection significantly reduced yield in every series except that receiving the full nutrient solution, NPK. Percentage reduction in yield due to the disease is highest in series NK⅓P and next in series ⅓(NPK).

Of particular interest is the fact that deficiencies of N and of K, which failed significantly to depress yield in the control series, nevertheless significantly reduced yield in the inoculated series. Also of interest is the fact that deficiency of P alone reduced the yield more severely, in both control and inoculated series, than the deficiency of all three nutrients together. Although with this particular scale of nutrients and nutrient deficiencies, phosphate deficiency produced the most striking reduction in yield, it would be unwise to stress too far the parallel between this experimental result and Australian observations. The chief conclusion to be drawn from this experiment is that any nutrient deficiency, if sufficiently severe, is likely to intensify the loss in yield through disease.

## SUMMARY

Red Marvel spring wheat plants were grown singly in sand culture in 7 in. glass flower pots, under conditions of full nutrient supply, and under deficiencies of nitrogen, phosphate and potash and of all three together, respectively. After one month's growth, the plants were inoculated with *Ophiobolus* by the insertion of two pieces of infected wheat straw into the sand on each side of and just below the crown.

Satisfactory root infection occurred in every plant inoculated, but was lightest in the nitrogen-deficient plants, whilst the potash-deficient plants exhibited a rather more intense root infection than those of any other series. Percentage infection of the stem-bases was lowest in the full nutrient and in the nitrogen-deficient plants, and highest in the series deficient in all three nutrients.

In the uninoculated control plants, a significant depression in grain yield was produced only by the deficiency of phosphate; in the inoculated plants, however, deficiency in any one of the three plant nutrients significantly reduced grain yield. Infection significantly reduced yield in every series except that receiving full nutrients; the percentage reduction was greatest in the phosphate-deficient plants.

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# EFFECT OF ADDITION OF SUGAR ON RATE OF DECAY OF WOOD

By W. P. K. FINDLAY, M.Sc.

*Forest Products Research Laboratory, Princes Risborough, Bucks*

It has sometimes been suggested that the presence of soluble food materials in the sap and of reserve carbohydrates in the parenchyma cells appreciably increases the liability of wood to decay. The seasonal variations in the content of these materials in wood has been put forward as an explanation of the differences in susceptibility to rot of timber felled at different seasons of the year, but Gäumann (1938) found that the time of year when the content of reserve food materials in pine and spruce wood is greatest does not coincide with the time when the wood is most readily attacked by fungi. He suggested that it is the presence of increased amounts of growth-promoting substances and the resulting variations in the physical condition of the cell walls which favour the development of fungi on wood felled during the period when the annual ring of wood is being laid down.

Strong solutions of sugar have, on the other hand, been suggested for use as wood preservatives, but the limited success obtained with the "Powellizing" process was probably due more to the arsenic which was usually incorporated with the sugar solution used for treating the wood than to the sugar itself. It is obvious that if a sufficiently high concentration of sugar is present it may render the wood unsuitable for the growth of micro-organisms by raising the osmotic pressure of any liquid in it above the point that can be tolerated by the organism.

Schmitz & Kaufert (1938) investigated the effect of the addition to sawdust of dextrose and of dextrose together with asparagine and concluded that the rate of decay of wood by certain fungi, e.g. *Lenzites trabes*, may be increased by the addition of small amounts of dextrose with or without asparagine. In the case of other fungi such as *Lentinus lepideus*, the rate of decay was apparently decreased by the presence of added nutrients.

In view of the conflicting nature of these results it appeared worth while investigating this problem further.

## Experimental

After oven-drying and weighing, small blocks of Scots pine (*Pinus sylvestris*) sapwood measuring $5 \times 2{\cdot}5 \times 1{\cdot}5$ cm. were completely impregnated by means of a vacuum pump with various concentrations of sucrose or dextrose. After reweighing they were then exposed to the air until the moisture content had dropped to about 35 % (of the dry weight). After sterilization at 15 lb. gauge pressure in an autoclave, the samples were exposed to fungal infection by being placed in cultures of various wood-rotting fungi growing actively on 2 % malt agar in special Kolle flasks. The samples were supported on small glass rests so that they came into contact only with the fungal mycelium and did not rest on the medium. After 5 or 10 weeks' exposure to fungal attack the samples were removed from the flasks, freed from adhering fungal mycelium, weighed, and after oven-drying, reweighed. This "final" dry weight subtracted from the initial dry weight gives the loss in weight due to fungal decay, which, expressed as a percentage of the original weight, is a useful measure of the amount of decay which has occurred.

Where samples are treated with solutions of sugar stronger than 0·5 % an error is introduced in the calculation of the final dry weight if an appreciable amount of sugar remains undestroyed in the samples at the end of the experiments. It was found that the blocks after exposure to attack did contain

fairly large amounts of sugar, so allowance was made for this by adding to the initial weights of the samples the weight of sugar absorbed, which was calculated from the amount of solution of known concentration taken up by the samples. The losses in weight were in every case calculated as a percentage of the original dry weight of wood alone. Ten samples at each concentration were exposed to each of the fungi so that the figures quoted in Tables 1 and 2 are the means of ten results.

## Results obtained

*Experiment* 1. (Table 1.)

The results shown in Table 1 indicate that the addition of small amounts of sucrose has little, if any, effect on the loss in weight of wood brought about by *Lentinus lepideus*, *Coniophora cerebella* or *Polystictus versicolor*. Where the concentration of the sugar solution was 5·0 % the loss in weight, *disregarding the weight of sugar absorbed*, was in each case less than that of the controls. If the weight of sugar be added to the initial weight of dry wood substance the

Table 1

| | | | Loss in dry wt. of samples as % of original wt. of wood | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0·5 % sucrosé | | 1·0 % sucrose | | 5·0 % sucrose | |
| Timber | Fungus | Period weeks | Control | No allowance for wt. of sugar | Wt. of sugar added to I.W. | No allowance for wt. of sugar | Wt. of sugar added to I.W. | No allowance for wt. of sugar | Wt. of sugar added to I.W. |
| Scots pine sapwood | *Lentinus lepideus* Fr. | 5 | 9·95 | 9·94 | 10·52 | 7·79 | 9·06 | 4·77 | 11·45 |
| | | 10 | 19·6 | 19·75 | 20·35 | 19·15 | 20·39 | 16·18 | 22·91 |
| | *Coniophora cerebella* Pers. | 5 | 14·9 | 14·95 | 15·52 | 13·00 | 14·38 | 10·17 | 16·98 |
| | | 10 | 26·7 | 26·79 | 27·18 | 24·82 | 26·09 | 22·60 | 29·22 |
| Beech | *Polystictus versicolor* (L.) Fr. | 5 | 12·92 | 14·32 | 14·79 | 14·13 | 14·86 | 10·13 | 15·01 |
| | | 10 | 23·62 | 22·94 | 23·44 | 24·71 | 25·63 | 20·57 | 25·28 |

total loss of dry weight (loss of sugar and wood) is only slightly greater than loss of wood alone. The loss cauesd by *Lentinus lepideus* after 5 weeks is very much less than half that caused in 10 weeks if we disregard the weight of sugar, but almost exactly half the 10 weeks' loss if the weight of sugar be taken into consideration. This indicates that during the early stage of attack this fungus is deriving its nourishment mainly from the sugar and leaving the wood practically unaffected.

*Experiment* 2. (Table 2.)

In this experiment the samples were treated with solutions containing various amounts of dextrose (Table 2).

Table 2

| | Loss in dry wt. % of Scots pine samples after 5 weeks | | | | |
|---|---|---|---|---|---|
| | | 1 % dextrose (hydrated) | | 2·5 % dextrose | |
| Fungus | Control | No allowance for wt. of sugar | Wt. of sugar added to I.W. | No allowance for wt. of sugar | Wt. of sugar added to I.W. |
| *Merulius lacrymans* (Wulf.) Fr. | 5·47 | 4·49 | 5·98 | 1·80 | 5·59 |
| *Coniophora cerebella* Pers. | 8·53 | 6·55 | 6·61 | 5·88 | 9·65 |
| *Poria vaillantii* (D.C.) Fr. | 5·51 | 4·13 | 5·62 | 2·11 | 5·87 |
| *Lenzites trabea* Pers. | 10·37 | 10·31 | 11·79 | 8·40 | 12·19 |

In every case the loss in dry weight of the samples, *disregarding the weight of added sugar*, was less than that of the controls. Where allowance is made for the full weight of sugar added, the total loss of dry matter caused by *Merulius lacrymans* and *Poria vaillantii* was not significantly greater than that of the controls: in the samples attacked by *Coniophora cerebella* and *Lenzites trabea*, the total loss was only slightly greater than in the controls. It is very difficult to determine what relative proportion of the loss has been due to utilization of the sugar as compared with destruction of wood substance because decomposition of the cellulose results in the production of sugars which would invalidate any attempt at analytical determinations of the sugar remaining after exposure to the fungi. If we assume, as seems reasonable, that at least half of the added sugar is destroyed by the fungi, it will be found that the estimated loss due to destruction of wood substance is *less* than in the controls.

## Discussion

There is no evidence from these results that the addition of dilute solutions either of sucrose or of dextrose stimulates the growth of the test fungi in such a way that appreciably greater amounts of wood substance are destroyed by them. Visual examination of the amount of fungal mycelium on the treated and on the control samples supports this view. On the other hand, there is an indication that certain fungi, such as *Lentinus lepideus*, *Coniophora cerebella*, *Merulius lacrymans* and *Poria vaillantii*, cause actually less destruction in the presence of added sugar. Probably these fungi utilize the readily available carbohydrates in preference to the more refractory polysaccharides of the wood, and after the former are consumed, decay will proceed at approximately the same rate as in untreated wood. For instance the loss in control blocks by *Coniophora cerebella* after 5 weeks was 4·7 % more than in those with 5 % sucrose (no allowance for sugar weight), and after 10 weeks 4·1 % more. Similarly, with *Polystictus versicolor* after 5 weeks controls had lost 2·8 % more than those treated with 5 % sucrose, and after 10 weeks 3·0 % more, i.e. the differences remain about the same and do not increase proportionately and any initial difference due to the attack falling on the sugars instead of the cell wall does not continue to affect *rate* of decay.

It is also clear from these results that treatment with a 5·0 % solution of sucrose does not have any appreciable *preservative* effect and that it would be necessary to use very much higher concentrations of sugar in order to raise the osmotic pressure of the solution sufficiently to inhibit fungal growth therein.

The results obtained in this investigation support Gäumann's view that variations in the content of soluble carbohydrates do not materially affect the liability of wood felled at different seasons to decay by wood-rotting fungi and it appears more likely that the differences which have been observed are due, as he suggests, to variations in the condition of the cell walls.

The results obtained by Schmitz & Kaufert (1938) were somewhat contradictory, *Lenzites trabea* causing more decay and *Lentinus lepideus* less decay in *sawdust* soaked in a solution of sugar than in sawdust moistened with water. It should be noted that these investigators used sawdust as a medium and did not experiment with solid wood, and also that only two test fungi were employed. It has been found that very much more vigorous growth is obtained in cultures on sawdust to which a small percentage of readily available carbohydrates has been added than on pure sawdust. In sawdust the presence of a film of nutritive solution will encourage the development of a vigorous growth of superficial mycelium, leading to the

rapid infection of the bulk of the material. Many wood-rotting fungi which attack solid wood vigorously make but feeble growth on sawdust, unless nutrients be added and a very high moisture content maintained in the sawdust. Fungi which flourish at moisture contents of 40–50 % in solid wood require moisture contents of 150–200 % in sawdust. All the physical conditions in a finely divided, well-aerated medium are very different to those existing in wood, therefore conclusions based on experiments with sawdust should be applied with reserve to solid wood.


## Summary

Experiments were carried out to determine whether the addition to wood of dilute solutions of sucrose or of dextrose increases its rate of destruction by various wood-rotting fungi. It was found that there is no significant difference in the amount of decay between samples treated with 0·5 or 1·0 % sucrose or with 1 % dextrose, and controls treated with water. The loss in weight of samples treated with 5 % sucrose or 2·5 % dextrose was less than that of the controls, but if allowance be made for the whole of the weight of sugar added, the total loss was slightly greater than that of the controls. It is believed that a large proportion of such loss is due to destruction of the sugar and that the destruction of wood substance is less in the presence of large amounts of readily available sugars, which are utilized by the fungus in preference to the more refractory polysaccharides in the wood.


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# SAMPLING FOR LEATHERJACKETS WITH ORTHODICHLOROBENZENE EMULSION

By H. F. BARNES, M.A., Ph.D.

*Entomology Department, Rothamsted Experimental Station, Harpenden, Herts*

(With 2 Text-figures)

A few years ago a long-term study of the yearly fluctuations in numbers of leatherjackets (the larvae of *Tipula paludosa* Meigen) was started. The method adopted was to obtain them by watering a lawn with an emulsion of orthodichlorobenzene. The St Ives Leatherjacket Exterminator, an emulsion of orthodichlorobenzene and Jeyes fluid, was used at normal strength, applying it at the rate of 1 gal./sq. yd. The leatherjackets come to the surface within a few minutes and are in no way permanently damaged. If not removed, they will later descend once more into the turf and soil. Adult craneflies were reared from the leatherjackets so obtained by placing them in pots of soil in which there was germinating wheat. By this procedure it was hoped to obtain figures for the relative parasitism (by *Bucentes* spp.) from year to year and the flight period of the cranefly as well as yearly fluctuations in the numbers of the leatherjackets themselves.

The site chosen was the strip of lawn between the path and hedge running roughly north and south in the home apiary at Rothamsted Experimental Station. In 1936 this area was divided into ten blocks of 8 sq. yd., 1 sq. yd. per block being sampled each week for 8 successive weeks.

An account of the first year's results was published by Barnes (1937), together with notes on methods of investigating the bionomics of the species.

In view of the importance of leatherjackets consequent upon the ploughing-up campaign, it has been thought desirable to give an account of the investigations from 1937 to 1940 inclusive, particularly as evidence has been collected regarding sampling for leatherjackets with orthodichlorobenzene emulsion.

While the sampling to be described has been carried out in the spring only, Miss Lovibond (1937*a*) sampled monthly from October onwards. It was found that there was a definite increase in numbers from October to November, but then the numbers were fairly constant till the spring. It is possible that this autumn increase is due to some of the young leatherjackets in the first two instars not appearing on the surface because such larvae may sustain permanent injury from the treatment. But in any case the error involved in finding the very small larvae must be great compared with that when they are larger and easily seen on the grass. Miss Lovibond found that by early October most of the larvae had reached their third instar.

Secondly, all the sampling described has been carried out on grassland. Tests have been made on arable land and the leatherjackets respond apparently just as well to the treatment. The only difference is one of application, i.e. along the rows of the crop rather than in square units.

The material required is a watering can with rose and a small bottle of the emulsion. One 100 c.c. bottle contains sufficient to treat 8 sq. yd. Water to mix with the exterminator can usually be obtained on the farm and can if necessary be taken from a ditch. The amount of emulsion necessary for 2 gal. of water is 25 c.c.

The long-term aspect of the problem will be mentioned incidentally, but emphasis will be laid more on the results bearing on the efficacy of such a sampling method. References to leatherjacket outbreaks will be found in a recent paper on the subject of sunspots and insect outbreaks (MacLagan, 1940).

The leatherjackets appearing on the surface of the turf were always removed and never allowed to re-bury themselves.

## Number obtained by weekly sampling

In 1936 the numbers of leatherjackets obtained in successive weeks fell steadily. It was thought that there should be a period during the winter and spring months during which the leatherjacket population remained practically constant. In the autumn great mortality would be expected to occur because it is known that the eggs and early instars of the larvae are very susceptible to drought. When once the larvae have assumed the typical leatherjacket

appearance (i.e. the third larval instar), the population would remain fairly constant until the next dangerous period in the life history had been reached. This would be when the presence of parasites made themselves felt, i.e. when they become fully grown and leave their leatherjacket host, in the late spring.

In 1937, instead of sampling for 8 successive weeks, the period was extended to 16 weeks, the area to be treated being divided into five blocks of 16 sq. yd.

TABLE 1. *Numbers of leatherjackets obtained from 5 sq. yd. of lawn per week*, 1937–40

(*Note.* The figures underlined represent the weeks before the regular fall in numbers set in)

| | 19–25 Feb. | 26 Feb.–4 Mar. | Mar. 5–11 | Mar. 12–18 | Mar. 19–25 | 26 Mar.–1 Apr. | Apr. 2–8 | Apr. 9–15 | Apr. 16–22 | Apr. 23–29 | 30 Apr.–6 May |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1937 | — | — | — | — | — | 143 | 163 | 143 | 137 | 73 | 57 |
| 1938 | — | 84 | 124 | 89 | 62 | 89 | 51 | 24 | 12 | 42 | 43 |
| 1939 | — | 225 | 191 | 203 | 184 | 132 | 100 | 80 | 62 | 36 | 38 |
| 1940 | 61 | 132 | 127 | 75 | 91 | 123 | 127 | 157 | 117 | 122 | 120 |

| | May 7–13 | May 14–20 | May 21–27 | 28 May–3 June | June 4–10 | June 11–17 | June 18–24 | 25 June–1 July | July 2–8 | July 9–15 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1937 | 41 | 17 | 15 | 9 | 3 | 6 | 4 | 1 | 2 | 2 |
| 1938 | 7 | 8 | 0 | 5 | 4 | 1 | — | — | — | — |
| 1939 | 17 | 10 | 3 | 4 | — | — | — | — | — | — |
| 1940 | 36 | 15 | 5 | 12 | 3 | — | — | — | — | — |

Table 1 gives the numbers of leatherjackets so obtained in the years 1937–40 inclusive. The dates of sampling have been varied from year to year in an attempt to obtain more data before the numbers of leatherjackets start to fall. In 1937, only 4 weeks' sampling revealed

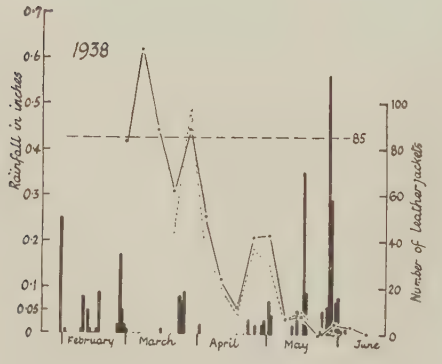


Fig. 1.

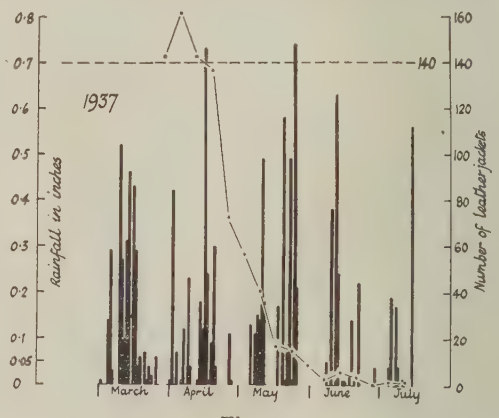


Fig. 2.

Fig. 1. Numbers of leatherjackets obtained in the weekly sampling and the daily rainfall, 1938. (The dotted curve represents the numbers obtained in a duplicate experiment by Mr D. C. Thomas.)

Fig. 2. Numbers of leatherjackets obtained in the weekly sampling and the daily rainfall, 1937.

a level population. In 1938, although the sampling dates were considerably advanced, only 5 weeks showed this level period. This was undoubtedly due to the inability of the emulsion (1 gal./sq. yd.) to penetrate sufficiently to reach the leatherjackets during the serious spring

drought of that year. Fig. 1 shows the effect of rain later causing the numbers to rise again temporarily. A comparison with Fig. 2 makes it obvious that even when there is abundant moisture there is a later falling off in the numbers of leatherjackets obtained by this method of sampling. In 1939, a somewhat less level period was found to occur for 4 weeks. In 1940 the sampling dates were again advanced and the first week's sampling was done while frost was still in the ground although not on the surface. The fourth and fifth weeks showed low numbers, but for 8 weeks the numbers obtained were approximately level.

Each year a steady decrease in the numbers of leatherjackets obtained took place after an initial period when the numbers were fairly constant. The dates on which this fall in numbers started varied from year to year.

The reasons for this decrease might be either that the leatherjackets at this period of the year failed to respond to orthodichlorobenzene treatment or that their numbers were becoming greatly and steadily reduced at this season of the year. Such a decrease might be due to parasitism, predators or a definite movement away from the area treated or a downward movement out of range of such treatment. The results of rearing craneflies from the leatherjackets in 1936, 1937 and 1938 showed that the percentage parasitism was almost negligible and could not possibly be held responsible. The other possibilities causing this steady decrease in numbers obtained each year are discussed later.

### Population per year

Table 2 gives the approximate population per 5 sq. yd. and per 1 sq. yd. for the years under consideration. These numbers are obtained from averaging the weekly figures before the yearly decrease set in.

Table 2. *Numbers of leatherjackets*

| | Per 5 sq. yd. | Per 1 sq. yd. |
|---|---|---|
| 1937 | 140 | 28 |
| 1938 | 85 | 17 |
| 1939 | 200 | 40 |
| 1940 | 125 | 25 |

### Results of a second treatment with orthodichlorobenzene

Lovibond (1937*b*) gave a table showing the results, obtained in 1937, of a second treatment of the emulsion at varying intervals after an initial treatment: the numbers obtained increased with the period after the initial treatment. This experiment was repeated in 1940 and the results of both these experiments are given in Table 3.

Table 3. *Yield of leatherjackets from 5 sq. yd. on second application of treatment*

| 29 Apr. 1937 | | 21 Mar. 1940 | |
|---|---|---|---|
| No. of days between 1st and 2nd treatment | No. of leatherjackets obtained | No. of days between 1st and 2nd treatment | No. of leatherjackets obtained |
| 3 | 21 | 2 | 27 |
| 10 | 29 | 8 | 35 |
| 17 | 43 | 16 | 65 |
| 24 | 54 | 21 | 43 |
| 31 | 74 | 29 | 52 |

The figure 65 in the 1940 experiment is probably accounted for by the chance that several rather heavily populated square yards occurred originally in the neighbourhood of those

treated that particular week. The numbers recovered at the second treatment bear a slight direct relationship to those originally obtained at the first treatment, higher figures at the first treatment being, in general, followed by higher figures at the second treatment.

These experiments show that there is a lateral movement either above or below ground. Ignoring the effect of such movement the standard of efficacy of the sampling is that not less than 80% of the leatherjackets are brought to the surface and found.

### Results of treatment on three successive days

In 1940 it was decided to treat certain square yards each day until the numbers obtained became constant and so would reveal the extent of daily immigration into the treated area. Unfortunately an area which was thinly populated was the only one available and the experiment could not be continued after the third day.

Ten sq. yd. were treated on each day from 2 to 4 Apr. inclusive. At otal of 130 leatherjacket were obtained the first day, thirty-one the second and eight on the third. Lateral migration appeared to be taking place into the treated square yards, as several of the leatherjackets on the second and third days appeared in close proximity to the borders impinging on untreated turf. Even if this immigration is ignored, the figures obtained indicate that 75·5% were brought up by the treatment on the first application, 19·5% on the second and 5% on the third.

### The yearly steady decrease in numbers of leatherjackets obtained by watering with orthodichlorobenzene

Barnes tentatively suggested (1937) that leatherjackets are not susceptible to orthodichlorobenzene treatment during the period of at least 6 weeks prior to pupation. If this were so, it would follow that periodic watering with orthodichlorobenzene would indicate the earliness or lateness of the season as regards pupation. Secondly, if this non-susceptible period represents a non-feeding prepupal period, a date should easily be ascertained each year after which baiting with paris green and bran would be ineffective.

In order to find evidence for a non-feeding prepupal period, fifteen leatherjackets, obtained by the orthodichlorobenzene treatment on 15 May 1940, were washed and kept isolated in Petri dishes containing germinating wheat. The wheat grains were examined daily and up to 30 May there was positive evidence that feeding was taking place almost daily. After this date the feeding became less regular but as late as 11 June the surviving nine leatherjackets were still feeding. On 17 Aug. there were five survivors; four of these pupated between 21 and 29 Aug. Two males emerged on the twelfth day after pupation, namely 4 and 9 Sept. The others died.

About the same time Mr H. C. Gough kept four leatherjackets, which had been obtained the following week, in soil with growing wheat between two sheets of glass and darkened on the outside. He reported that till about the end of May each leatherjacket accounted for one seedling of wheat per night but that during June they fed much less voraciously. Towards the end of July the solitary survivor started feeding more actively again.

A third experiment with five leatherjackets, which had been obtained by digging after they had failed to respond to orthodichlorobenzene treatment on 28 May, indicated that they were still feeding on 12 June, some time after becoming non-susceptible to such treatment. Four of these pupated between 21 and 27 Aug., three males emerging between 30 Aug. and 8 Sept. Pupation in these cases lasted between 9 and 13 days.

Another experiment was carried out in an attempt to discover whether the leatherjackets obtained by digging, together with those few responding to emulsion treatment late in the season, accounted for the total original population. Accordingly, 1 sq. yd., previously untreated, was treated on 28 May and only four leatherjackets were brought up. When the turf and soil were removed to a depth of 7 in. and examined, ten leatherjackets were found in the turf layer and none below. Thus fourteen was the total number of leatherjackets obtained. The adjacent square yards when treated on 9 and 23 Apr. produced fifty-seven and forty-five respectively and it is apparent that some of the leatherjackets originally in the yard under discussion had not been found. Therefore these two adjacent square yards were treated on 30 May to see if lateral movement could account for the missing leatherjackets. The result of this test was that only three and two leatherjackets were brought to the surface. These numbers are lower than would be expected (see p. 25) if all the leatherjackets still responded to treatment. The next step was to discover if the leatherjackets had moved from the lawn into the rough grass alongside. Two, seven and nil leatherjackets were obtained from the 3 sq. yd. immediately adjacent to the 3 sq. yd. of lawn under investigation, while two, five and nil were brought up in the 3 sq. yd. further in the rough grass. A cinder path bounds the lawn on the other side. It is apparent that the numbers obtained cannot account for all the leatherjackets originally present in this area. Some of them may have moved right out of the area either above ground[1] or less probably below ground or else they may have descended in the soil deeper than 7 in. which is very improbable.

Various experiments have been carried out to ascertain how often and when leatherjackets respond to orthodichlorobenzene treatment. Leatherjackets once brought to the surface by this treatment, allowed to rebury and then immediately treated a second time would not respond the second time, but if a few hours (5–6) or weeks were allowed to lapse then the leatherjackets would respond the second time.

Finally, five leatherjackets which had failed in the field to respond to a primary treatment and had been obtained by digging were subjected to a second treatment after a day or two. (It is possible but unlikely that they were not reached by the emulsion on the first occasion.) The second treatment failed to cause any of them to come to the surface although care was taken to see that the emulsion was sure to reach them.

In conclusion, it would appear that at a certain stage, before they have finished feeding and a long time before pupation takes place, leatherjackets become non-susceptible to orthodichlorobenzene. In addition, about this time but not necessarily at exactly the same time, there is a considerable movement, probably above ground, somewhat similar to the wandering period exhibited by certain caterpillars after they have become fully fed and before they are ready to spin their cocoons and pupate.

## Conclusions

The orthodichlorobenzene method is satisfactory for estimating rapidly the numbers of leatherjackets present early in the year, providing it is used on suitable days, i.e. damp rather than dry ones, warm rather than frosty ones. About 80% of the leatherjacket population is obtained.

It is unreliable later in the season when the leatherjackets are approaching their fully fed stage. For one reason the leatherjackets appear to become immune to orthodichlorobenzene

[1] Mr A. Roebuck informs the writer that he has seen a large overground movement of leatherjackets.

and, secondly, at this period a definite movement away from the original feeding site appears to take place.

The dates when the treatment becomes ineffective vary from year to year. Generally speaking it is effective till the end of March, but it may be used some years throughout April with good results.

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# SOME OBSERVATIONS ON THE LIFE HISTORY OF THE ANGLESHADES MOTH (*BROTOLOMIA METICULOSA* L.)

By W. J. PARR and E. R. SPEYER, M.A.
*Experimental and Research Station, Cheshunt, Herts*

(With Plate 3)

The Angleshades moth is common and widely distributed in Europe and is often an inhabitant of glasshouses, especially of those devoted to cultivation of chrysanthemums. The caterpillars are very destructive to flowers of chrysanthemum, and at least in the later instars prefer petals to foliage, though they have been reared with ease entirely upon the latter. They feed voraciously upon leaves of cineraria and geranium, and caused much injury to those of broad beans grown under glass during the winter of 1939–40.

Staniland & Beaumont (1935) refer to depredations upon anemones grown for market, Wilson (1931) gives rhododendron, Theobald (1927) chickweed, dock, groundsel, primrose, and violet, and Staniland & Beaumont (1935) add bracken and broom as outdoor food plants. Other hosts of economic importance recorded from the continent of Europe are apple, artichoke, beet, cabbage, celery, hop, lettuce, pear, strawberry, sunflower, and vine; Enser (1938), quoting Kaltenbach, gives fifteen outdoor hosts, including *Lamium*, *Mercurialis*, *Rosa*, and *Urtica*.

Caterpillars kept in the laboratory at Cheshunt completed their metamorphoses upon chrysanthemum, geranium and cineraria, but would not feed upon hydrangea or *Polygonum*. Enser (1938) records severe injury to cyclamen under glass in Austria.

## Seasonal history

In British glasshouses, caterpillars are to be found usually only during the autumn and winter months: it would appear that eggs are laid upon chrysanthemum plants before they are brought into the houses in late summer and early autumn, and that the majority of pupae derived from this generation are removed with the pot-soil when the crop is ended in January or February. Another generation is produced on outdoor plants in late spring and early summer. Staniland & Beaumont (1935) state that moths occur in May and June, and again in September. Chevalier (1927) also records two generations annually in France.

## Life history

(1) *Oviposition*. Little reference can be found to observations upon deposition of eggs, though Theobald (1927) states that the eggs are laid singly or in groups upon leaves, and that they hatch in 2 weeks. Moths of both sexes which emerged from pupae kept in a heated laboratory during January and February 1939 were caged with suitable plants and sugary solutions upon which to feed: in no instance were any eggs laid. Pupae kept at much lower temperatures during the winter of 1939–40 emerged on 20 Mar.: one female and three males were kept together in a large cage containing a cineraria plant until 1 Apr. and then removed to a small one containing a chrysanthemum plant and some geranium leaves in

water. During the night 112 eggs were deposited, ninety being scattered over the muslin covering to the cage, six laid singly upon the upper, and twelve in, at most, groups of two upon the lower surface of chrysanthemum foliage, the majority at or near leaf tips; and three upon the underside of geranium leaves: at least fifty more were laid in a similar manner by this female during the following night upon the cover of the cage. The eggs adhere firmly to the surface upon which they are deposited.

(2) *The eggs.* The eggs (Pl. 3, fig. 1) were spherical, slightly depressed, pale greenish yellow and radially sculptured, with a diameter of about 0·75 mm. Two to three days after deposition, a brownish ring appeared round the upper portion of each. The great majority hatched on 12 Apr., giving an incubation period of 10 days at room temperature.

(3) *The larva.*

First instar: length, at hatching, 3 mm.; at first the head is extraordinarily large in proportion to the rest of the body. Colour, yellowish to dirty green; transverse rows of setae raised on very prominent black tubercles. Second instar: length, immediately after ecdysis, 5 mm. Colour, green: three narrow broken white stripes dorsally, a white stripe laterally on each side: only a few setae raised on dark tubercles. Third instar: length, immediately after ecdysis, 12 mm. Colour, green; dorsal stripes as in second instar, lateral stripes bordered by a darker green stripe: integument covered with minute white specks, setae not raised on tubercles, but a white speck at their origins. Intersegmental areas yellowish. Fourth instar: length, immediately after ecdysis, 17 mm. Colour as third instar, but lateral white stripes less conspicuous. Fifth instar: length, immediately after ecdysis, 21 mm. Colour, green: pairs of oblique grey markings dorso-laterally; lateral stripes dark grey: nine pairs of oval spiracles very prominent, white, ringed with black. Sixth instar: length, immediately after ecdysis, 28–34 mm. Colour, dark green, or light brown, with dark oblique markings: a single broken white stripe dorsally, lateral stripe as in fifth instar. Intersegmental areas reddish brown. When full grown the caterpillar measures about 1½ in.

The above notes upon colour changes refer to a small number of caterpillars bred in the laboratory: from collections of larvae made in chrysanthemum houses it is apparent that the colours are exceedingly variable (Pl. 3, fig. 3), some assuming a brown tint at least as early as the third instar, while others are almost white in the early instars, and become bright green or reddish brown only in the later instars. From the third instar onwards, caterpillars may be distinguished from those of the commoner Noctuids by their smooth velvety appearance.

The usual number of instars appears to be six, but two caterpillars, which were eventually reared to moths of each sex, completed seven instars prior to pupation.

On hatching from the egg, the young larvae eat the egg shell, and the greater portion of the cast skin, usually with the exception of the head capsule, is devoured at each ecdysis, a fact which explains the absence of moulted skins from plants upon which they are living. In the first instar the larvae hang from the foliage on threads if disturbed, and they remove the epidermis principally from the upper side of the leaves (Pl. 3, fig. 2). Already, in the second instar, extensive holes are eaten in foliage, or the surface of chrysanthemum flower-buds may be removed, this causing subsequent deformation of the petals. Feeding takes place at night, and during the daytime the caterpillars lay hidden in curled leaves, amongst the petals of flowers, or, in the case of broad bean, amongst the leaves in the vicinity of the growing point. It was noted that during cold spells, caterpillars were inclined to expose themselves upon the upper surface of bean leaves.

Some larvae of the spring generation were bred at least as far as the pupal stage, and the duration of the various instars, which showed considerable variability under laboratory conditions, is given in Table 1.

TABLE 1. *Spring brood: eggs laid, 1–2 Apr. 1940; hatched, 12 Apr. 1940*

| | Duration of instars in days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Larva no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Av. |
| First and second instars | 12 | } 24 | } 21 | } 19 | — | } 17 | } 22 | } 20 |
| Third instar | 8 | | | | | | | |
| Fourth instar | 4 | | 4 | 5 | — | 3 | 2 | 4 |
| Fifth instar | 8 | 9 | 14 | 8 | — | 6 | 9 | 9 |
| Sixth instar | 6 | 11 | 18 | 12 | — | 10 | 14 | 12 |
| Seventh instar | 12 | 5 | — | — | — | — | — | — |
| Total | 50 | 49 | 57 | 44 | 40 | 36 | 48 | 46 |
| Prepupa | 10 | 11 | 6 | 8 | 12 | Died | Died | 9 |
| Pupa | 20 | 19 | Died | 19 | 14 | — | — | 18 |
| Date of emergence | 1 July | 1 July | — | 22 June | 17 June | — | — | — |
| Sex | ♂ | ♀ | — | ♀ | ♀ | — | — | — |

Theobald (1927) states that the larvae feed during a period of 5–7 weeks, and that the moths emerge 2–4 weeks after pupation.

By keeping caterpillars in small boxes, it was possible to obtain cast head capsules, which were seldom devoured with the rest of the moulted skins; measurements of the breadth of these, given in Table 2, were made to determine if they conform to the principles of Dyar's law.

TABLE 2. *Measurements in mm. of breadth of cast head-capsules. Average ratio of increase for larvae nos. 3, 4, 6, 7 = 1·55*

| | | Observed | | | | | |
|---|---|---|---|---|---|---|---|
| Instar | Calculated | No. 1 | No. 2 | No. 3 | No. 4 | No. 6 | No. 7 |
| First | — | 0·44 | 0·44 | 0·44 | 0·44 | 0·44 | 0·44 |
| Second | 0·68 | 0·78 | — | — | — | — | — |
| Third | 1·05 | 0·91 | — | 1·07 | — | 1·10 | 0·93 |
| Fourth | 1·63 | 1·41 | 1·52 | 1·55 | 1·50 | 1·60 | 1·50 |
| Fifth | 2·53 | 1·95 | 2·36 | 2·32 | 2·34 | 2·45 | 2·37 |
| Sixth | 3·92 | 2·64 | 2·91 | * | 4·50 | 3·43 | 3·23 |
| Seventh | 6·08 | 3·71 | * | — | — | — | — |

* Denotes that the capsule was obtained but could not be measured owing to breakage.

It is apparent that the measurements for the larvae with six instars (nos. 3, 4, 6, 7) correspond fairly closely with the calculated figures, except perhaps those of the last instar. Larvae 1 and 2 certainly moulted seven times, and deviation from the calculated measurements indicate that this is not normal for the species. Numerous heads of first instar larvae were measured, and their breadth was invariably 0·44 mm.

(4) *The pupa.* When fully fed, the caterpillars descend to the ground, and bury themselves to a depth of about 1 in.: they spin a somewhat loose cocoon, into the thin fabric of which particles of soil are woven (Pl. 3, fig. 4). The chrysalids themselves are chestnut brown in colour, and measure about $\frac{3}{4}$ in. in length (Pl. 3, fig. 5). The average period spent as a pupa is about 18 days for the spring generation (see Table 1) and is preceded by a prepupal stage of variable duration.

For the winter generation the pupal period is much longer: during the winter of 1938–9 pupae were kept in a warm room, during that of 1939–40 in a cold room, and the results are tabulated in Table 3.

Chevalier (1927) gives 3 months as the pupal period, and Enser (1938) obtained pupae in early December, from which the first moth emerged on 30 Jan.

TABLE 3. *Duration of pupal instar: winter generation*

| Food plant | Larva pupated | Emergence | Sex | Pupal period days |
|---|---|---|---|---|
| Chrysanthemum | 21–24. xi. 38 | 9. i. 39 | ♀ | 47 |
| | 24. xi. 38 | 18. i. 39 | ? | 53 |
| | 30. xi. 38 | 23. i. 39 | ♂ | 52 |
| Cineraria | 22. i. 39 | 27. ii. 39 | ♀ | 36 |
| | | | | Months (approx.) |
| Broad beans | x. 39 | ii. 40 | ♂♀ | 3½ |
| | x. 39 | 20. iii. 40 | 5♂♂♀ | 5 |

(5) *The imago.* The moths (Pl. 3, fig. 6) are active only at night and do not appear to be attracted to light. They feed readily upon sugary solutions, but were not attracted to "moth-jars" containing beer and treacle, as employed for trapping tomato moths, when these were hung in chrysanthemum houses during the winter months: during spring and summer, however, they are sometimes found in the jars, though only in very small numbers.

Moths which emerged from pupae in January 1939 could not be induced to breed: a female lived for 14 days, and a male for 26 days: another male which emerged on 30 Jan. was still alive on 3 Mar. It appears that the moths are long-lived, and that a considerable period elapses between emergence and oviposition.

## PARASITES

Nielsen (1917) records the Tachinid, *Winthemia quadripustulata* F., in Denmark.

A prepupa and a pupa, out of considerable numbers obtained from caterpillars in commercial chrysanthemum houses at Cheshunt during the previous autumn, each yielded an adult of the Tachinid, *Phryxe vulgaris* Fall. at the end of January 1939.

Caterpillars sometimes, but rarely, served as a host of the Eulophid, *Comedo opaculus* Thoms. (Speyer, 1934).

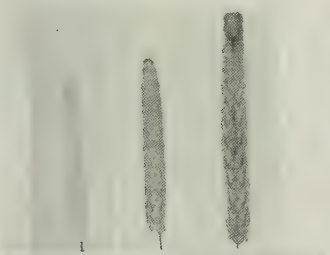
## CONTROL MEASURES

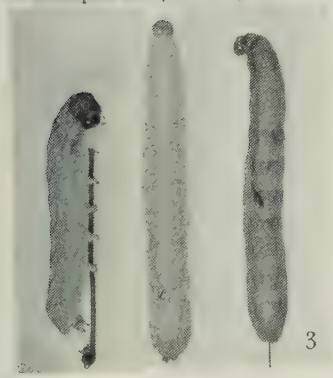
(*a*) *Lead arsenate.* Used in the form of a paste at the rate of 6 lb. to 100 gal. of water (or 1 oz./gal.) lead arsenate is most effective in killing caterpillars feeding upon foliage. It cannot, however, be employed for chrysanthemum plants at the time of flowering and these plants should therefore be sprayed immediately after they are brought under glass in autumn: further applications at intervals of 3 weeks may be necessary, but should cease 3 weeks before the first blooms are ready for market.

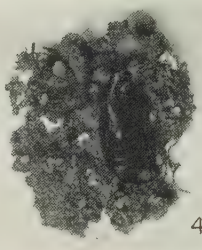
(*b*) *Cryolite.* When concealed amongst the petals of chrysanthemum flowers, which are ruined by the application of sprays, the caterpillars are most difficult to destroy, except by the very laborious and unsatisfactory method of hand-picking. If spraying with lead arsenate has been neglected until too late, powdered cryolite may be lightly dusted on to the blooms. This mineral has proved to be a most effective stomach poison, and has been the means of killing the caterpillars concealed even in large flowers, before they have removed appreciable numbers of the petals, within a period of 6 days after a single application. Attempts have been made to obtain a suitable "filter", although the mineral itself does no damage to the blooms, and a mixture of 1 part cryolite to 4 parts finely powdered pumice would appear to offer some possibility of success.

(*c*) *Derris powder.* Experiments with derris powder showed that the foliage and flowers of chrysanthemum were rendered distasteful to caterpillars, which, however, were able to







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abstain from feeding for a period of at least 9 days. Application of the powder on a large scale would not be likely to meet with success. Proprietary sprays containing the powder proved useful in preventing destruction of foliage in the case of vegetables to which application of poisonous substances was undesirable.

A brand of pyrethrum dust which had proved of great value in control of aphides and thrips proved useless in preventing caterpillars from feeding, and also had no contact action upon them: there can be little doubt that they actually consumed the powder without any ill effect to themselves.

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## EXPLANATION OF PLATE 3

*Brotolomia meticulosa* L. on chrysanthemum

Fig. 1. Eggs laid on foliage. × 1.
Fig. 2. Foliage of same plant showing injury from caterpillars a week after hatching. × ½.
Fig. 3. Half and fully grown caterpillars. × 1.
Fig. 4. Pupal cocoon opened to show chrysalis. × 1.
Fig. 5. Emerged pupae. × 1.
Fig. 6. Moths. × 1.

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# THE FEVER FLY, *DILOPHUS FEBRILIS* L., AND METHODS FOR CONTROL OF ITS LARVAE IN CULTIVATED LAWNS

By E. E. EDWARDS, M.Sc.
*University College, Cardiff*

Lawns continually mown are sometimes seriously disfigured by brown patches due to larvae of the fever fly, *Dilophus febrilis* L., feeding at the roots of the plants. According to Curtis (1844, 1860), the fly was given this name by Linnaeus because of the widespread belief at the time in Sweden that it occurred in houses where intermittent fevers existed. There is, however, no definite evidence to connect the fly with any human disease, and it is now generally recognized that its apparent association with outbreaks of cholera and other fevers was merely a coincidence.

Although there are references in the literature to damage done by larvae of the fever fly to various cultivated plants (Curtis, 1860; Theobald, 1910, 1913; Lovibond, 1938), it is generally believed that these grubs are mainly harmless, feeding principally on decaying matter. It is often claimed that the presence of these larvae in turf is therefore associated with a high proportion of decaying matter in the surface layers, or, more often, with the liberal use of farmyard manure by itself or mixed with soil in the form of a compost. In the latter instances, it is generally assumed that the eggs are laid by the flies either in the dung prior to its use as a top dressing or later in the turf soon after the application. Infestations are not, however, confined exclusively to swards unusually rich in organic matter, or to those recently manured. On several occasions in South Wales in 1938–40, sports turf and private lawns very low in humus and known to have received no organic manure or compost for many years were observed by the writer to suffer acutely from attacks by the fever fly. For example, at the Port Talbot Golf Course, Glamorgan, which suffered severely in the early spring of 1939, the larvae were present in great numbers not only on the putting greens but also on the approaches and fairways as well as on the isolated patches of natural herbage endeavouring to become established on the sand dunes adjoining the links. In all cases, the humus content of the soil was exceptionally low and no organic fertilizer had been applied on any part of the golf course for several years. The injury produced by the attack was particularly serious on the putting greens and distinctly greater there than on the other areas, presumably due to the poorer root system which resulted from repeated cutting of the sward.

## Symptoms of attack

In turf, the damage may be general or, more commonly, concentrated at first in relatively small areas varying in size from a few inches to a foot or more in diameter. These patches are irregular in shape, the grasses in the centre appear dead or dying, while those on the margin are yellow and only partially destroyed. A sod showing these symptoms contains immense numbers of larvae, often as many as 3500 or more to 1 sq. ft., and the turf is soft and spongy in character due to the activity of the grubs in loosening the soil around the roots. The larvae normally occur at a depth of not more than $\frac{1}{2}$ in. below the surface and in large colonies or "nests".

In serious cases of attack, the affected patches increase very rapidly in size and often merge into one another until finally extensive areas, several yards in diameter, become affected. The

damage produced by heavy infestations is most acute in turf on very sandy soil, in a poor state of fertility, and continually mown, as with tennis lawns and golf or bowling greens. The most probable explanation of this fact is the poorer root development consequent on regularly severe defoliation, and the lack in the soil of adequate supplies of readily available nutrients essential for rapid plant growth under conditions of continual mowing.

In lighter infestations, when the damage is marked chiefly by local thinness of the grass, the swards usually recover in superficial appearance but are liable to become infested with weeds and with an abnormal proportion of *Poa annua*, an undesirable grass on bowling greens and other cultivated lawns. Turf on heavier types of soil is seldom seriously affected by the insect.

## Life history

The adult flies appear in late April or early May, according to the locality and prevailing climatic conditions; warm, dry weather favours their early emergence. They are very active in bright sunshine, visiting the flowers of cultivated and wild plants, as well as those of trees and bushes. They are often seen in numbers frequenting the blossoms of apple, pear, cherry and raspberry, and are probably valuable pollinating agents. Towards dusk and in dull weather they become exceedingly sluggish, resting among herbage or on foliage of trees and bushes.

Pairing takes place soon after emergence and each female lays several hundred eggs in clusters, sometimes on, but more frequently just under, the surface of the ground. Eggs are also deposited in decaying vegetable matter, particularly round the edges of heaps of grass mowings. The period of incubation varies from 18 to 25 days, and the larvae remain together, often forming large colonies several inches in width. These colonies are usually found about $\frac{1}{2}$ in. beneath the surface, at least when occurring in firm, established turf. In about 3 months, largely depending on the weather, the larvae become fully fed and then burrow to a depth of 2–4 in. below the surface where each larva constructs for itself an oval cell within which it turns into a pupa. The depth at which pupation occurs varies according to the texture and moisture content of the ground, being approximately 4 in. in well-drained soils of a sandy character. From these pupae adult flies emerge after 3 weeks, that is, towards late August or September. Eggs are laid in similar locations to those selected by the first brood of flies and a further generation of larvae is produced.

The larvae of the second brood remain in this stage and continue to feed close to the surface, whenever the weather is not frosty, until the following April, at least under the conditions which prevailed in south Wales in 1939. Pupation then takes place at a depth similar to that of the first generation and some 3 weeks later the adult flies hatch out ready to start the cycle of operations of another season.

From observations made in 1939 there are reasons for supposing that all the larvae of the first generation do not always reach maturity in the autumn but continue their feeding until the following April when they pupate, the adult flies emerging about 3 weeks later. The subject requires, however, further investigation extending over several seasons under varying soil conditions before final conclusions can be reached.

## Field control trials

Since no treatment has hitherto been known for controlling the larvae of the fever fly with any certainty of success when occurring in turf, an opportunity was taken of the severe outbreaks in south Wales and Monmouthshire in the early spring of 1939 to study this problem. Experiments designed

to destroy the larvae were conducted under field conditions at three different centres, namely, (*a*) golf greens at Port Talbot, Glamorganshire, (*b*) tennis lawns at Cyncoed, Cardiff, and (*c*) bowling greens at Abergavenny, Monmouthshire.

Five substances were tested at each centre on plots arranged in randomized manner. Each experimental plot had an area of 72 sq. ft. and at each centre a group of three plots was taken as a unit for any one treatment. Care was exercised that all the plots, including the three controls, at each of the three centres were as uniform as possible in extent of infestation by the larvae, judging by the damaged condition of the sward.

*Chemicals tested*

The following five chemicals were applied to the respective plots at the rates given below:

(1) *Calomel dust.* This preparation contained 4 % mercurous chloride. It was evenly spread over the ground at the rate of 3 oz. to each plot and watered in, using 1 gal./sq. yd.

(2) *Orthodichlorobenzene emulsion.* This emulsion was prepared according to the formula described by Dawson (1932):

| | |
|---|---|
| Orthodichlorobenzene ... ... ... ... | 2 pt. |
| 10 % sodium oleate solution ... ... ... | ½ pt. |
| Jeyes's fluid ... ... ... ... ... ... | ½ pt. |

The ingredients were stirred together in a small container until the mixture reached the consistency of a thick paste when an additional 2½ oz. of Jeyes's fluid were added. For use, the emulsion was diluted at the rate of ½ pt./25 gal. of water to give a fluid containing 1 % orthodichlorobenzene. This diluted emulsion was applied at the rate of 1 gal./sq. yd. of turf.

(3) *Lead arsenate powder.* This material was uniformly distributed over the ground at the rate of 12 oz. to each plot and washed into the soil, using 1 gal./sq. yd.

(4) *Derris powder* (2 % rotenone). 10 oz. of this powder was thoroughly mixed with 24 gal. of water to give a solution with a rotenone content of 0·0054 %. It was applied at the rate of 1 gal./sq. yd.

(5) *Derris and pyrethrum emulsion.* 10 oz. of derris powder (2 % rotenone) and 38·5 fl. oz. pyrethrum extract (0·1 % pyrethrin I) were thoroughly mixed with 24 gal. of water. This fluid therefore contained 0·0054 % rotenone and 0·001 % pyrethrin I, and it was applied at the rate of 1 gal./sq. yd.

All these insecticides were applied at Port Talbot on 5 Apr. and at the two other centres on the following day. The weather was mild and calm and the ground in a fairly moist condition. All the larvae were in the fourth instar and most of them occurred at a depth not exceeding ½ in. below the surface of the ground. The actual application of the insecticides was done personally by the writer so as to ensure uniformity of treatment. The control plots received no special treatment except that they were given an equivalent quantity of water to that used when applying the different insecticides on the other plots, being at the rate of 1 gal./sq. yd.

## Discussion of results

A few days after the application of the insecticides, estimations were made of the numbers of live and dead larvae present in both treated and control plots. The technique adopted for this purpose consisted in taking six samples of the turf, with a circular hole-cutter, at random over each plot, each sample measuring 4 in. in diameter and 3 in. in depth. The samples were taken to the laboratory and the number and condition of the larvae present determined. The results are tabulated in Table 1, and the figures given in columns 4–6 represent the average number of larvae found in the six turf samples examined from each plot. It is evident from the figures for the total number of larvae (column 4) present in the turf of the different plots that the degree of infestation was fairly uniform throughout the whole experimental area at each centre. A further outstanding feature shown by these figures is the very high population of larvae in the experimental areas, especially at Port Talbot where the average number of grubs for all plots (column 4) was 3368 per sq. ft. or, approximately, 147 millions/acre.

The figures for the average percentage of larvae killed, shown in the final column of Table 1, indicates that all the insecticides tested, with the exception of calomel dust (plots 2),

TABLE 1. *Effect of different treatments on larvae of* D. febrilis

| Centre | Treatment | Serial no. of plot | Total no. of larvae | No. of live larvae | No. of dead larvae | Av. % of larvae killed |
|---|---|---|---|---|---|---|
| Port Talbot | Untreated (control) | 1*a* | 302 | 302 | 0 | |
| | | 1*b* | 214 | 314 | 0 | Nil |
| | | 1*c* | 282 | 282 | 0 | |
| | Calomel | 2*a* | 291 | 291 | 0 | |
| | | 2*b* | 327 | 327 | 0 | Nil |
| | | 2*c* | 278 | 278 | 0 | |
| | Orthodichlorobenzene | 3*a* | 267 | 70 | 202 | |
| | | 3*b* | 253 | 61 | 182 | 74·7 |
| | | 3*c* | 295 | 75 | 225 | |
| | Derris | 4*a* | 270 | 11 | 259 | |
| | | 4*b* | 296 | 18 | 278 | 94·7 |
| | | 4*c* | 298 | 17 | 281 | |
| | Derris-pyrethrum | 5*a* | 289 | 6 | 283 | |
| | | 5*b* | 315 | 9 | 306 | 97·9 |
| | | 5*c* | 273 | 3 | 270 | |
| | Arsenate | 6*a* | 323 | 0 | 323 | |
| | | 6*b* | 291 | 0 | 291 | 99·7 |
| | | 6*c* | 309 | 3 | 306 | |
| Cardiff | Untreated (control) | 1*a* | 241 | 241 | 0 | |
| | | 1*b* | 265 | 265 | 0 | Nil |
| | | 1*c* | 250 | 250 | 0 | |
| | Calomel | 2*a* | 275 | 275 | 0 | |
| | | 2*b* | 239 | 239 | 0 | Nil |
| | | 2*c* | 247 | 247 | 0 | |
| | Orthodichlorobenzene | 3*a* | 251 | 69 | 182 | |
| | | 3*b* | 271 | 80 | 191 | 69·4 |
| | | 3*c* | 263 | 91 | 172 | |
| | Derris | 4*a* | 238 | 17 | 221 | |
| | | 4*b* | 264 | 24 | 240 | 91·9 |
| | | 4*c* | 255 | 20 | 235 | |
| | Derris-pyrethrum | 5*a* | 258 | 2 | 256 | |
| | | 5*b* | 240 | 3 | 237 | 98·7 |
| | | 5*c* | 266 | 5 | 261 | |
| | Arsenate | 6*a* | 244 | 1 | 243 | |
| | | 6*b* | 251 | 2 | 249 | 99·6 |
| | | 6*c* | 246 | 0 | 246 | |
| Abergavenny | Untreated (control) | 1*a* | 183 | 183 | 0 | |
| | | 1*b* | 189 | 189 | 0 | Nil |
| | | 1*c* | 206 | 206 | 0 | |
| | Calomel | 2*a* | 198 | 198 | 0 | |
| | | 2*b* | 179 | 179 | 0 | Nil |
| | | 2*c* | 173 | 173 | 0 | |
| | Orthodichlorobenzene | 3*a* | 164 | 44 | 120 | |
| | | 3*b* | 184 | 49 | 135 | 73·2 |
| | | 3*c* | 201 | 54 | 147 | |
| | Derris | 4*a* | 198 | 12 | 186 | |
| | | 4*b* | 190 | 11 | 179 | 94·9 |
| | | 4*c* | 167 | 5 | 162 | |
| | Derris-pyrethrum | 5*a* | 188 | 3 | 185 | |
| | | 5*b* | 179 | 2 | 177 | 98·9 |
| | | 5*c* | 192 | 1 | 191 | |
| | Arsenate | 6*a* | 205 | 0 | 205 | |
| | | 6*b* | 183 | 2 | 181 | 99·6 |
| | | 6*c* | 185 | 0 | 185 | |

had been used with advantage at all centres. The most successful results followed the application of lead arsenate (plots 6) when, on an average for all centres, 99·6 % of the larvae were destroyed. The use of derris, either alone (plots 4) or in conjunction with pyrethrum extract (plots 5), also proved highly effective at the concentrations tested, the average percentage mortality at the three centres being 93·6 for the former and 98·5 for the latter. Although orthodichlorobenzene emulsion (plots 3) showed a marked toxicity towards the larvae, nevertheless it did not, in the circumstances described, exert a control equal to that derived from the use of either lead arsenate or derris preparations, the average percentage killed being 72·5.

None of the insecticides tested adversely affected the herbage. On the contrary, all the plots which were dressed with lead arsenate, derris and pyrethrum, derris alone, and orthodichlorobenzene showed within a few days of treatment a remarkable improvement in the appearance of the sward, due undoubtedly to the lethal action of these preparations upon the larvae feeding at the roots of the plants.

Although the use of lead arsenate at the rate of approximately 10 lb./1000 sq. ft. or 4 cwt./acre proved, under the conditions of the present experiments, a successful means of destroying the larvae of *D. febrilis* in turf, there are objections to its use on account of its poisonous properties. Equally efficient control for all practical purposes was obtained by the use of derris preparations which are non-poisonous to human beings and domestic animals. The exact minimum rotenone content that is necessary in a diluted solution of derris to give the desired results cannot be definitely stated but it would seem that a concentration of 0·0054 % is sufficient, when applications are made at the rate of 1 gal./sq. yd. Such a solution is readily prepared by adding, to every 38 gal. of water, 1 lb. of derris powder with a guaranteed rotenone content of 2 %.

## Summary

1. Symptoms of attack by larvae of the fever fly, *Dilophus febrilis* L., in lawns under conditions of continual mowing are presented, together with a brief description of the life history of the insect.

2. An account is also given of field experiments in 1939 at three different centres with calomel, orthodichlorobenzene, lead arsenate and derris preparations, for the control of the larvae when occurring in cultivated lawns.

3. Lead arsenate at 1½ oz./sq. yd., and also a derris-pyrethrum mixture (0·0054 % rotenone and 0·001 % pyrethrin I) used at 1 gal./sq. yd. gave almost complete control. A liquid derris preparation alone (0·0054 % rotenone) gave on the average about 95 % control and an emulsion containing 1 % orthodichlorobenzene about 72 % control, both applied at 1 gal./sq. yd. A 4 % calomel dust at ⅜ oz./sq. yd. was ineffective.

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# OBSERVATIONS ON THE LIFE HISTORY OF *ANGUILLULINA PRATENSIS*

By C. H. GADD and C. A. LOOS
*Tea Research Institute, St Coombs, Talawakelle, Ceylon*

(With 4 Text-figures)

*Anguillulina pratensis* is an important pest of tea bushes growing in certain Ceylon soils. It destroys the small feeding roots and causes lesions in the cortex of the larger woody roots. Large colonies of eelworms in all stages of development are to be found at the junction of living and dead tissues of the lesions, and it was from such places that the worms used in the following experiments were collected.

## Population within woody tea roots

Pieces were cut from the roots of an attacked bush so that each piece consisted of wood and cortex from the perimeter of lesions. After separating the cortex from the wood the pieces were left overnight in a gauze sieve in a funnel of water. The following morning a small volume of water was drawn off into a watch glass as is usual with the Baermann technique. In order further to concentrate the nematodes, the water in the watch glass was taken up in a pipette, and after about half an hour, during which time the worms accumulated at the lower end, drops were collected on microscope slides and the worms in each classified and measured. The observations are summarized in Table 1, sample 1 from tea cortex.

The specimens forming sample 2 were collected on another occasion from another tea bush. After the cortex was separated from the wood the worms on the cambial surface were washed off in a small quantity of water, which was then taken up in the vertical tube and the worms concentrated as before (see Table 1, sample 2).

In both samples, the ratio of larvae to adults is very low, and there is no statistically significant difference between the two results. That there should be only one larva to every 2·6 adults indicates a much slower rate of propagation than is normal with many other species of nematodes, unless the method used favoured the collection of adults in preference to larvae. Both adults and larvae are very sluggish, and no difference in their movements in water is noticeable. It is probable that, in both samples, only those worms which were free on the cambial surface were collected, and consequently, the ratios observed may not be truly representative of the ratio within the cortical tissue. Later experiments showed that the rate of multiplication is greater than is indicated by these ratios.

There were no statistically significant differences between the ratio of males to females or between the mean length measurements of larvae and of females in the two samples. The males of sample 2, however, were significantly smaller than those of sample 1. The difference between the means with its standard error is $25 \pm 9{\cdot}3\,\mu$. The frequency distributions of the length measurements of larvae, males and females collected from tea roots are shown in Fig. 1.

Measurements (summarized in Table 1) were also made of 100 eggs collected from tea cortex, and of twenty-three larvae recently emerged from eggs hatched in damp sand in the laboratory. The eggs were in various stages of development; thirty-six were in an advanced

stage and had a mean measurement of 66·2 × 28·8 μ as compared with the mean of 62·7 × 26·2 μ of the other sixty-four. As the differences are of statistical significance it indicates that there is an enlargement of the egg during development.

Table 1. *Measurements of* Anguillulina pratensis *from tea roots and soil*

| | No. measured | Min. μ | Max. μ | Mean μ | Standard deviation μ |
|---|---|---|---|---|---|
| From tea roots: | | | | | |
| Larvae, lengths: | | | | | |
| Sample 1 | 49 | 206 | 432 | 315 | 60·9 |
| Sample 2 | 62 | 206 | 477 | 328 | 72·2 |
| Samples 1 and 2 | 111 | 206 | 477 | 323 | 66·8 |
| Males, lengths: | | | | | |
| Sample 1 | 58 | 422 | 645 | 567 | 51·4 |
| Sample 2 | 49 | 398 | 645 | 542 | 44·8 |
| Samples 1 and 2 | 107 | 398 | 645 | 555 | 49·8 |
| Females, lengths: | | | | | |
| Sample 1 | 92 | 336 | 755 | 560 | 94·7 |
| Sample 2 | 91 | 394 | 748 | 578 | 68·0 |
| Samples 1 and 2 | 183 | 336 | 755 | 569 | 74·5 |
| Eggs: | | | | | |
| Length | 100 | 54 | 76 | 63·8 | 4·50 |
| Width | 100 | 20 | 36 | 27·1 | 3·29 |
| Larvae, newly hatched: | | | | | |
| Length | 23 | 208 | 282 | 243 | 17·89 |
| Width | 23 | 11·2 | 15·6 | 13·1 | 1·42 |
| From soil: | | | | | |
| Lengths: | | | | | |
| Larvae | 106 | 216 | 480 | 331 | 68·7 |
| Males | 35 | 308 | 581 | 463 | 58·7 |
| Females | 54 | 416 | 662 | 548 | 54·8 |

## Population from soil

*A. pratensis* occurs in the soil surrounding the roots of infected bushes. The worms in the thicker roots tend to move inwards to the cambium and away from the dead tissue, not outwards to the soil. It is therefore considered that the worms found in the soil are mainly those which have left dead feeding roots and are migrating in search of other roots.

Specimens were collected from the soil by the Baermann method. About 100–200 g. of soil were placed in a gauze sieve (60 mesh) about 4 in. in diameter in a funnel of water. It is advisable to place the sieve in the funnel before adding the water, which should be poured in slowly to avoid disturbing the soil, otherwise, an excessive number of soil particles accumulates at the lower end of the funnel and the separation of the worms becomes difficult. All the worms are not removed from the soil by this method, but it was considered that those collected would be fairly representative of the soil's content. A summary of the observations made from several soils from one estate is given in Table 1.

The ratio of larvae to adults (106 : 89) is higher than that found in the root samples. No great importance can be attached to this observation as the soil samples varied so markedly amongst themselves. The ratio of males to females (1 : 1·5) was very similar to that observed in the populations from roots (1 : 1·7). There is no significant difference between the mean measurements in length of larvae from the soil and from roots, but both males and females found in the soil are on the average smaller than those from roots.

## EXPERIMENTS *IN VITRO*

*Egg laying*. Attempts were made to maintain females in water and saline solutions in watch glasses and hanging drops, and on plain agar (3%) in Petri dishes, in order to ascertain the number of eggs laid by one individual and the frequency of laying. We were, however, unable to maintain the worms under these conditions for more than 6 or 7 days, and usually egg laying ceased after 2 or 3 days. As a rule, one egg only is to be seen in the uterus at any time and that egg is usually laid within 24 hr. The greatest number of eggs obtained from any one worm, before death occurred, was eight in 6 days. The usual rate of laying was one per day though occasionally some worms laid two eggs during the day.

In one experiment the females were placed in agar hanging drops. They soon left the agar and deposited their eggs in the moisture condensing on the cover glass around the agar drop. One laid four eggs in 3 days before it died.

The conditions of the experiments were obviously unsatisfactory.

*Egg hatching*. Eighty-five eggs in various stages of development were collected from the cortex of attacked tea roots and placed in hanging drops, or drops of water placed on microscope slides. Of these, only five hatched, i.e. 6%, during the 3 weeks they were kept under observation. The longest observed period for incubation was 11 days. It is not suggested that that is a maximum or even a normal incubation period, as the age of the eggs at the beginning of the experiment was unknown. A somewhat higher percentage hatch, viz. 19%, was obtained from ninety-eight eggs, selected as being in an advanced stage of development, when placed under similar conditions. In other experiments the eggs were placed on plain agar in Petri dishes, but only four hatched out of fifty-six, i.e. 8%. No better results were obtained when the agar was used as hanging drops on cover glasses.

The very low percentage hatch in these experiments was possibly due to two major adverse factors: (1) injury to the eggs during manipulation, and (2) unsatisfactory aeration. Possible injury to the eggs can be avoided by inducing the worms to lay their eggs in a position where they can be observed without further manipulation; and an improvement of aeration can be obtained by using damp sand as a medium instead of fluids or agar.

Mention has already been made of a female that laid four eggs in 3 days in a hanging drop. These eggs and many others laid under similar conditions were kept under observation for lengthy periods, but three of this four were the only ones to hatch. Two hatched after 13 days and one after 12 days.

Newly laid eggs were obtained by placing female worms in a small quantity of water or other fluid in watch glasses or on microscope slides, and leaving them overnight. Later, the worms were removed with a fibre needle, the eggs counted, and the receptacle placed in a damp chamber for further observations. In this way it was possible to observe the eggs without any risk of injury to them by manipulation. With the exception of those mentioned above no eggs were observed to hatch in fluids. It appeared probable, therefore, that aeration was the more important factor in limiting incubation.

Better results were obtained when damp sand was used as a medium. The sand was a river sand which would pass through a 60-mesh wire sieve. It was thoroughly washed until the supernatant fluid was clear, and then dried. Newly laid eggs were obtained in water on microscope slides as already described, and sufficient dry sand was then added to absorb the water. No attempts were made to standardize the relative amounts of water and sand used; the sand was poured on to the slide until all the water was absorbed and the sand formed a small damp mound over the eggs. The slides were then placed in damp chambers and examined at intervals.

For examination, the sand was washed off the slide into a watch glass with a small pipette. The same water was taken up in the pipette and squirted through the sand several times. In this way the sand was well washed and blown to one side; then the water was again taken up, but this time it was placed in another watch glass for examination under a low-power microscope. The numbers of larvae and eggs were then counted. The sand was washed in this way three times, unless all the eggs known to be on the slide could be accounted for earlier. As a rule the bulk of the larvae and eggs were obtained in the first washing, and the third one usually contained neither eggs nor larvae. It was not always possible to account for all the eggs, but it is considered improbable that many larvae were not found. Each mound of sand was examined once only.

No larvae were found till the 12th day, and the records for the 12th day onwards are shown in Table 2. The results are given as a fraction, the numerator being the number of larvae found, and the denominator the number of eggs originally seen on the slide before the sand was added. The

Table 2. *Hatching of newly laid eggs of* Anguillulina pratensis *in damp sand*

| Days | | | | | | | | | | | | Total | % hatched |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 3/5 | 0/1 | 0/2 | 0/1 | 0/1 | 0/3 | 0/1 | 0/9 | 0/4 | | | 3/27 | 11·1 |
| 13 | 0/1 | 0/1 | 1/1 | 0/1 | 4/6 | 1/3 | 0/2 | 1/4 | 0/3 | 0/1 | 1/3 | 8/26 | 30·8 |
| 14 | 1/1 | 0/1 | 0/1 | 1/3 | 3/3 | 0/5 | 0/2 | 0/6 | 0/1 | 0/3 | 0/2 | 5/28 | 17·8 |
| 15 | 0/1 | 0/10 | 2/4 | 3/4 | 2/3 | 1/3 | 0/4 | | | | | 8/29 | 27·6 |
| 16 | 0/1 | 0/1 | 4/7 | 1/4 | 0/5 | 0/7 | 2/3 | 1/3 | | | | 8/31 | 25·8 |
| 18 | 9/15 | 0/4 | 1/3 | 4/7 | | | | | | | | 14/29 | 48·3 |

results, however, are so inconsistent that it is not possible to draw other conclusions than that a minimum incubation period is 12 days. No satisfactory indication is given of the optimum period.

A somewhat similar experiment was carried out with eggs of unknown age, as obtained from tea cortex. After the cortex was washed with water the eggs were taken up from the washings in a fine pipette. The pipette was held vertically for a few minutes to allow the eggs to settle and then a drop was released to a microscope slide. Any worms found amongst the eggs on the slide were then removed with a fibre needle. After counting the eggs, sand was added to absorb the water and the slides were then placed in damp chambers for later examination, as before. The results are tabulated in Table 3, and shown graphically in Fig. 2. As the eggs on each slide were of various ages it was to be expected that the numbers of larvae found on successive days would increase to a maximum after which time the number would remain constant.

Table 3. *Hatching of* Anguillulina pratensis *eggs from tea cortex in damp sand*

| Days | 3 | 4 | 5 | 6 | 7 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hatch | 2/49 | 6/38 | 14/58 | 9/41 | 14/22 | 14/41 | 11/31 | 23/37 | 30/55 | 21/48 | 10/19 |
| As % | 4·8 | 15·8 | 24·2 | 21·9 | 26·9 | 34·1 | 35·5 | 62·2 | 54·6 | 43·8 | 52·6 |

The maximum number of larvae was found on the 11th day (62 % of the eggs used), but that result and those of the following days are not consistent with those of the preceding days. The straight line which best fits the observation made from the 3rd to 10th day inclusive is $y = 0{\cdot}299 + 3{\cdot}66x$. If the line is prolonged it passes through $y = 51{\cdot}5$ when $x = 14$ days. The actual observation at 14 days was 52·6 %. If, however, a straight line is calculated from the last three observations the line obtained ($y = 37{\cdot}33 + x$) is by no means a good fit. From this, it would appear that the observations during the first 10 days were fairly consistent but the later ones are not.

The eggs used in this experiment were of different ages. If samples contained equal proportions of eggs of various ages, the graph representing the hatchings would be expected to be similar to that shown in Fig. 2 (as $y = 0{\cdot}299 + 3{\cdot}66x$) until the full incubation period is passed when the line would become horizontal. The line $y = 37{\cdot}33 + x$ representing the data obtained from observations made on the 12th, 13th and 14th days is more nearly horizontal, but because the fit is so poor and it intersects the other graph near the 14th and last day of the experiment no great importance can be attached to it. Moreover, the data from the observations made on the 13th and 14th day fit the line $y = 0{\cdot}299 + 3{\cdot}66x$ as well if not better than they do the line $y = 37{\cdot}33 + x$. We are therefore of the opinion that this experiment was stopped too soon, i.e. before the maximum period of incubation was passed and that the observations made on the 11th and 12th days were abnormally high, possibly due to the eggs in these samples being on the average somewhat older than those of other samples.

The experiment showed that a higher percentage of eggs will hatch in damp sand than in fluids and that satisfactory aeration is essential for the hatching of *A. pratensis* eggs. Doubtless, gravid females do lay eggs in the soil during their migrations from one root to another, but such layings are more or less accidental. The experiment was not repeated because it was found that more reliable data could be obtained from experiments using living roots, the normal medium of egg deposition of this species.

## Experiments with living roots

*Method.* After preliminary investigations the following methods were adopted. *Tephrosia Vogelii* was selected as the host plant because the roots were readily attacked by *Anguillulina pratensis*, seedlings at a suitable stage of growth were easily obtainable, and the roots were very satisfactory for later examination. The temperature of the laboratory in which these and the foregoing experiments were carried out and in which the growing plants were tended varied from 64 to 83° F.

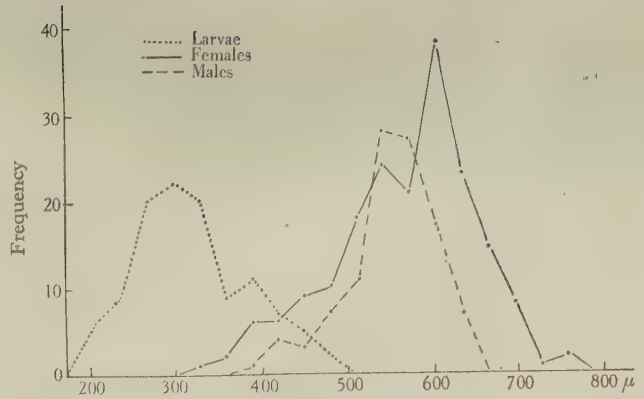


Fig. 1.

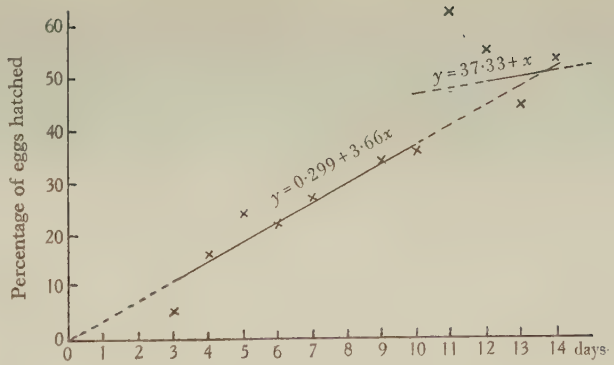


Fig. 2.

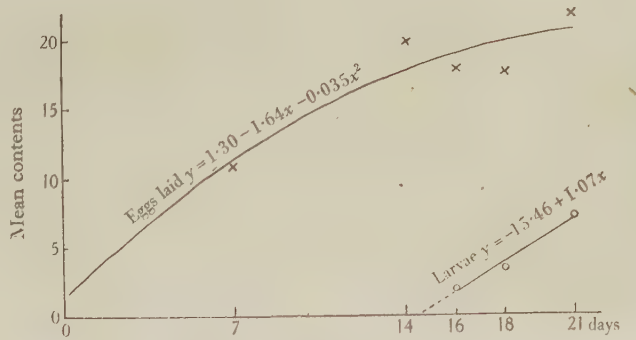


Fig. 3.

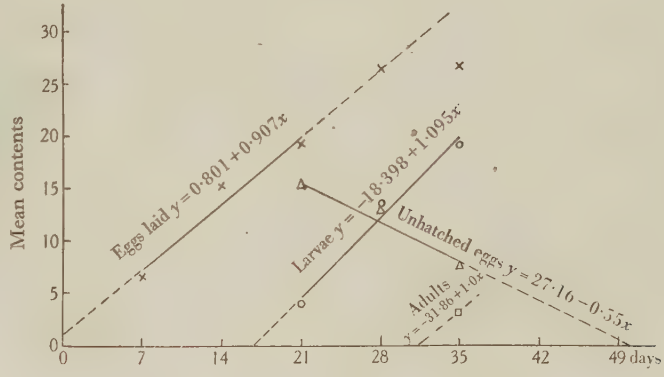


Fig. 4.

Fig. 1. Frequency distributions of length measurements of males, females and larvae of *Anguillulina pratensis* from tea roots.

Fig. 2. Time of hatching *Anguillulina pratensis* eggs from tea roots.

Fig. 3. Mean rate of egg laying in *Tephrosia Vogelii* root by a population of gravid females of *Anguillulina pratensis* from tea roots and the mean rate of emergence of larvae.

Fig. 4. Mean rate of egg laying in *Tephrosia Vogelii* by laying females of a population of *Anguillulina pratensis* from tea roots, and the mean rates of emergence of larvae, of their development into adults and of the cessation of egg laying.

(1) *Infection.* A drop of water was placed on a microscope slide or other piece of glass in a damp chamber and a solitary female worm was transferred to it with a fibre needle. A seedling, with a radicle about 1 in. long, was then placed on the glass so that its root tip rested in the water. After 24 hr. the seedling was removed and planted in sand in a flower pot. By this method about 65% of the seedlings used became infected, each by one eelworm. Infection by recently hatched larvae was obtained in a similar manner. When infection by one larva only is not required, a somewhat simpler method is to place eggs in damp sand as previously described. After a few days, depending upon the number of eggs in the sand and the number of larvae desired to infect a root, the root tip of a growing seedling is buried in the sand for 24 hr. before planting in a pot for further growth. In this way several seedlings may be infected from one lot of eggs, whereas if the sand is washed out to obtain single larvae no further use can well be made of the unhatched eggs.

(2) *Examination.* Both adults and larvae usually enter the seedling root a short distance behind the tip. By recording the length of root at the time of infection the approximate position of the parasite can be determined when the root has to be examined. A faint discoloration can be observed at the infected region 3 weeks after infection, and the discoloration is more marked 2 weeks later. The part of the root believed to contain the worm is removed, washed and then placed in a macerating fluid consisting of equal parts of 10% nitric acid and 10% chromic acid. The length of time the root is left in the macerating fluid depends upon the thickness of the root, but 1–2 hr. is usually sufficient. After maceration, the condition of the root should be such that, when placed on a microscope slide and covered with a cover glass, the root can be squashed into a thin layer by slight pressure on the cover glass. If the root is somewhat thick and woody, it is advisable to remove the woody cylinder after maceration has progressed for about 1 hr. The cortex is then replaced in the fluid for further maceration. Microscopic examination for eelworm content is still further facilitated if the root is well washed in water after maceration and then mounted in 10% caustic potash before squashing under the cover glass.

After entry into the root, neither the female nor the larvae travel far. Usually the female is surrounded by the eggs she has laid. If she moves, her track is easily recognizable by the trail of eggs left behind. Consequently, there is little difficulty in counting her offspring accurately. There was no evidence of the vagrant habit of *A. pratensis* mentioned by Steiner (1934). In four roots only, of the several hundreds examined, were eggs found without the female which laid them being in the immediate vicinity: it is probable that they left the roots after laying a few eggs.

*Series* 1: *gravid females.* In the first series of experiments only females with an egg visible in the uterus were used for infection—such females will, for brevity, be referred to as gravid. Very often, however, the egg which was visible was deposited in the water before the worm entered the root. The reason for selecting such females was to ensure that only worms actively laying eggs would be used. It did not, however, ensure that the worms would continue to lay eggs even for 1 week. On examination later, it was found that a few (five of the fifty-seven causing infection) laid no eggs, and others very few, whereas the maximum number found was forty-three after 21 days. It was evident, therefore, that some of the worms selected were near the end of their egg-laying period at the time they infected the roots. A summary of the results of examination made after 7, 14, 16, 18 and 21 days is given in Table 4 and Fig. 3.

Table 4. *Series* 1. *Gravid females of* Anguillulina pratensis *in* Tephrosia Vogelii *roots*

| | No. of ♀♀ observed | Layers | Mean (of layers) | Standard deviation |
|---|---|---|---|---|
| Eggs laid: 7 days | 11 | 11 | 10·7 | 3·35 |
| 14 days | 13 | 13 | 19·7 | 7·52 |
| 16 days | 12 | 10 | 17·7 | 7·13 |
| 18 days | 10 | 8 | 17·4 | 11·70 |
| 21 days | 12 | 11 | 21·5 | 11·92 |
| Eggs hatched: 16 days | 12 | 10 | 1·9 | 1·96 |
| 18 days | 10 | 8 | 3·4 | 4·84 |
| 21 days | 12 | 11 | 7·2 | 5·72 |

*Series* 2: *unselected females.* In the second series of experiments females picked up with a fibre needle under a simple dissecting microscope were used for infection whether an egg was visible in the uterus or not. No conscious selection was made, but it is probable that, subconsciously, larger

individuals were more frequently chosen because they would be easier to pick up and less likely to be larvae. The worms were examined with a microscope to ensure that only adult females had been selected before the *Tephrosia* roots were introduced. In all 547 worms were used, of which 351 or 64% entered roots. Examinations were made after 1, 2, 3, 4 and 5 weeks and are summarized in Table 5 and Fig. 4.

Table 5. *Series* 2. *Unselected females of* Anguillulina pratensis *in* Tephrosia Vogelii *roots*

| | No. of ♀♀ observed | No. dead | No. layers | Mean (of layers) | Standard deviation | Females with larvae | Females with adults | Females with unhatched eggs |
|---|---|---|---|---|---|---|---|---|
| Eggs laid: 7 days | 64 | — | 48 | 6·4 | 3·229 | — | — | — |
| 14 days | 73 | — | 63 | 15·0 | 7·358 | — | — | — |
| 21 days | 71 | 14 | 57 | 19·1 | 9·518 | — | — | — |
| 28 days | 65 | 11 | 54 | 26·3 | 12·031 | — | — | — |
| 35 days | 67 | 13 | 55 | 26·6 | 12·711 | — | — | — |
| Eggs hatched: 21 days | 71 | — | 57 | 3·9 | 3·139 | 50 | — | — |
| 28 days | 65 | — | 54 | 13·5 | 6·884 | 52 | — | — |
| 35 days | 67 | — | 55 | 19·2 | 10·133 | 53 | — | — |
| Adult offspring: 35 days | 67 | — | 55 | 3·1 | 2·741 | — | 43 | — |
| Eggs not hatched: 21 days | — | — | 57 | 15·1 | 8·221 | — | — | 53 |
| 28 days | — | — | 54 | 12·8 | 8·519 | — | — | 51 |
| 35 days | — | — | 55 | 7·4 | 6·924 | — | — | 40 |

*Series* 3: *larvae.* Larvae hatched in damp sand were used for infection of seedling roots, and so the exact age from hatching was not, as a rule, known. Some were not more than 2 days old and others might have been as old as 12 days from hatching. None, however, had had an opportunity of feeding on any root before entry into the *Tephrosia* seedlings. Many roots contained several larvae which did not wander far after entry, and so were usually found fairly near together when examined later. After egg laying commenced, it was not always easy to determine which individual had laid eggs. Roots were examined after 7, 14, 18 and 21 days, and 4, 5, 6 and 8 weeks, and the observations made are summarized in Table 6. Adult males were easily recognized, but it was not always possible to

Table 6. *Development of larvae of* Anguillulina pratensis *in* Tephrosia Vogelii *seedling roots. Roots infected by larvae recently emerged from eggs*

| Period of observation days | No. of larvae used | Females | Males | Larvae | Eggs | Max. no. of egg layers |
|---|---|---|---|---|---|---|
| 14 | 40 | 2 | — | 38 | — | — |
| 16 | 52 | 27 | — | 25 | — | — |
| 18 | 52 | 38 | 2 | 12 | — | — |
| 21 | 29 | 26 | 1 | 2 | — | — |
| 28 | 32 | 30 | 2 | — | — | — |
| 35 | 68 | 52 | 16 | — | 97 | 26 |
| 42 | 45 | 37 | 8 | — | 151 | 24 |
| 56 | 12 | 9 | 3 | 9 | 9 | 1 |

distinguish adult females from large larvae. In cases of doubt, if the worm exceeded 0·42 mm. in length it was regarded as an adult and if below that measurement as a larva. Measurements of 217 larvae from tea cortex recorded earlier included only twenty-two, or roughly 10%, which exceeded 0·42 mm., and of 237 females only thirteen or 5·5% were smaller than 0·42 mm. Actually, there were very few borderline cases.

## Discussion of results

*Non-layers.* Tables 4 and 5 show that a number of female worms which entered the roots did not lay eggs during the period of observation: they had either not reached the egg-laying stage or had completed it before entry into the roots. The worms originally came

from a population with a large proportion of males, and it seems fair to assume that the chances of picking out unfertilized females for these experiments were small. In series 2, after 1 week, 25% of the worms had laid no eggs; after 3 weeks the percentage of non-layers was still 25, and after 5 weeks 18% were non-layers. It appears probable, therefore, that the bulk of the non-layers consists of worms which have completed their egg laying, otherwise a reduction in their number would be expected at the later examinations, when the less mature worms would have begun to lay. In all, 340 females were observed of which sixty-three (18·5%) were non-layers. When the observed numbers are compared with the expected values on the hypothesis that there is no decrease with time, a $\chi^2$ value of 2·52 is obtained ($P$ lies between 0·5 and 0·7), from which it may be concluded that there is no reason to doubt the hypothesis. The means and standard deviations given in Tables 4 and 5 have, therefore, been calculated on layers only; non-layers are excluded from the observations.

In the samples taken at the end of 3, 4 and 5 weeks in series 2, a number of worms were seen which from their appearance were considered to have been dead before the roots were macerated. In all, thirty-eight dead worms were found, of which thirty-five (92%) had laid no eggs before death; the remaining three laid few eggs after entry into the root. In series 1, one worm was found dead at the end of the 2nd week, having laid one egg only, and four more were found in the samples examined during the 3rd week, none of which had laid eggs.

It is to be expected that deaths will occur at some time in the roots, but the fact that the majority of dead worms found within the roots died without laying eggs is of interest, though it is impossible to determine exactly when they died. Possibly they had completed their egg laying and death followed in the natural course of things. On the other hand, it is possible that they were injured in some way during manipulation, which injury resulted later in death. To enter a root, the worm punctures a small hole through the cell wall and then squeezes itself through the aperture. It seems improbable, therefore, that a worm injured mechanically during manipulation would succeed in doing this, which suggests that the majority of dead worms observed had completed their egg-laying period. Whether there is but one egg-laying period or a succession, with rests between, will be discussed later.

*Mean rate of egg laying.* In Fig. 4 the mean number of eggs found at each examination of series 2 is indicated by a cross. The straight line which best fits the data collected after 7, 14 and 21 days is represented by the equation $y = 0{\cdot}801 + 0{\cdot}907x$ and is shown in the figure as a solid line. Its prolongation is shown as a broken line which passes very close to the mean obtained from data collected at the end of the 4th week, but deviates markedly from the result of the 5th week's observations. This suggests that the rate of egg laying of this population was uniform till the end of the 4th week, after which time the rate diminished markedly. The mean rate of egg laying for the first 4 weeks is therefore 0·9 egg per day.

The rate of egg hatching will be a reflexion of the rate of egg laying so long as the incubation period remains constant. In Fig. 4 are also shown the mean numbers of larvae found after 3, 4 and 5 weeks as small circles. The straight line which best fits these observations is $y = -18{\cdot}398 + 1{\cdot}095x$, which indicates that the mean rate of egg hatching was 1·09 per day. This figure is in close agreement with that obtained for egg laying, viz. 0·9 egg per day.

The data obtained at each examination of series 1 are similarly represented in Fig. 3. In this series of experiments the rate of egg laying diminished in the 3rd week, as there is no statistically significant difference between the results obtained after 14 and 21 days. This perhaps is to be expected as the worms were specially selected as having started egg laying and, on the average, were more likely to be nearer the end of their egg-laying period than the unselected worms of series 2. The line which best fits the observed data is a quadratic parabola with the formula $y = 1{\cdot}30 + 1{\cdot}64x - 0{\cdot}035x^2$. This suggests a higher mean rate of egg laying during the first few days than was found in series 2. An explanation of this observation is offered later.

The straight line which best fits the mean numbers of larvae found after 16, 18 and 21 days is that represented by $y = -15{\cdot}46 + 1{\cdot}07x$. This indicates that the mean rate of hatching and, consequently, of laying is 1·07 eggs per day, which is in close agreement with that derived from observations of series 2.

We may therefore conclude that the mean rate of egg laying of a mixed population of females is approximately one per day.

*Rate of egg laying of individuals.* It will be realized that the mean rate of egg laying of a population gives little indication of individual performances. The mean rate of a population over a period will normally be less than the mean rate of any individual unless all lay throughout the period. During the 1st week in series 1, eleven female eelworms laid 118 eggs, giving an average daily rate of 1·53 per worm. Of these eleven, one laid four, one laid five, and one laid nine. It seems probable that these worms ceased egg laying before the end of the week. The remaining eight worms laid in the 7 days from eleven to fourteen eggs with a mean of 1·78. This figure is probably fairly representative of the rate of egg laying of individuals. The maximum rate observed was two per day.

Another estimate of the mean rate of egg laying of individuals can be obtained from series 2. The number of eggs laid varied from one to fourteen during the 1st week. The frequency distribution observed is not incompatible with the hypothesis that the worms began to lay at regular intervals through the week, i.e. theoretically, there should be as many individuals with 1, 2 or 3 eggs as with 12, 13 or 14 ($\chi^2 = 13{\cdot}8$; $P$ lies between 0·30 and 0·50). The mean number of eggs was 6·4 which, when divided by 4 (the arithmetic mean of $1 + 2 + \ldots + 6 + 7$ days), gives a value of 1·6 per female per day. It should be mentioned here that the observed frequency distribution is more nearly a normal distribution than the above hypothesis suggests. When the observed results are compared with those calculated from a theoretic normal distribution $\chi^2$ has a value of 9·2, giving a probability lying between 0·8 and 0·9.

The value (1·6) given above as the mean number of eggs laid per female per day is very similar to that of the $x$ term (1·65) of the quadratic parabola formula derived from the egg-laying data of series 1. In series 1 all the worms laid eggs during the first few days though some apparently ceased before the end of the week. So long as $x$ remains very small the term $-0{\cdot}035x^2$ is negligible. Then the term $1{\cdot}64x$ expresses the mean rate of egg laying during the first few days when all worms were laying. The mean rate of egg laying for individual females is therefore approximately 1·65 eggs per day.

The maximum rate of egg laying observed was 2·4 eggs per day over a period of 2 weeks. The maximum number laid by one individual in 5 weeks, the longest period observed, was sixty-four, which, if she started laying at the beginning of the experiment, gives a mean of 1·8 per day.

*Incubation period.* It has already been shown that *in vitro* eggs may hatch in as short period as 12 days. Eggs deposited in roots take somewhat longer to hatch. The roots i series 1 and 2 contained no hatched eggs at the examination made after 14 days. Larva were observed in some roots of series 1 examined after 16 days. The eggs from which thes larvae originated were probably laid shortly after the females entered, in which case th incubation period as observed lies between 14 and 16 days.

The mean incubation period may be derived from Figs. 3 and 4. If the larval graphs ar prolonged until they cut the $X$-axis the points of intersection are 14·4 and 16·8 days fo series 1 and 2 respectively. It is probable that the estimate obtained in this way fro series 1 is an underestimate, as the slope of the line from which it was derived ($1{\cdot}07x$) less than that of the linear part of the egg-laying parabola ($1{\cdot}64x$) with which theoreticall it should be parallel. We would conclude therefore that the mean incubation period fo *A. pratensis* eggs under the conditions of these experiments is between 15 and 17 days.

*Larval period.* Adults other than those used for infection were observed for the firs time at the end of the 5th week in series 2. The mean number found was 3·14. Had th experiment been continued for a longer period the mean number of adults would hav increased in each succeeding week, and it is to be expected that the graph of such result would have been approximately parallel with those already shown in Fig. 4 for eggs an larvae. A dotted line representing the equation $y = -31{\cdot}86 + 1{\cdot}0x$ has been drawn to pas through the observed value 3·14 at 35 days. This line cuts the $X$-axis at 31·9 days. It i evident therefore that the mean larval period determined from these data is 15·1 days.

A more direct estimate of the larval period can be obtained from the results (Table 6 obtained from experiments of series 3 in which roots were infected with recently hatche larvae. The majority (93 %) became adult during the 3rd week and 52 % of them becam adult within 16 days. We may therefore conclude that the mean larval period is 15–16 days which is in close agreement with the conclusion drawn from series 2.

*Pre-egg-laying period.* Although the larvae in series 3 became adult in about 16 days eggs were not observed until the end of the 5th week, when ninety-seven eggs were found It was not possible to determine with certainty the number of females concerned in the laying of these eggs because some roots were infected by several larvae (eight was the maximum observed) and these congregated in such a way that it was often impossible to determine to which worm the eggs belonged. When those roots which contained no eggs were excluded, it became evident that the eggs had been laid by at most twenty-six females, giving a mean of 3·73 eggs per worm.

Using the formula $y = 0{\cdot}80 + 0{\cdot}91x$ obtained from the egg-laying observations in series 2 it is evident that a mean of 3·7 eggs would be reached in 3·2 days. It appears probable, therefore, that egg laying began about the 31st day (i.e. 35–3·2 days). This gives a period of about 15 days (i.e. 31 less a larval period of 16 days) between the larvae becoming adult females and the beginning of egg laying.

*Sex ratio.* Of the 318 larvae observed to become adult, only thirty-two (10 %) became males. This percentage is in marked contrast with that (37 %) found in naturally infected tea roots. Tyler (1933) found a very small percentage of males in single-nematode cultures of *Heterodera marioni*, whereas the percentage increased markedly in multiple primary infestations and in secondary galls. She concluded that the increase in percentage of males is correlated with the increasing adverse conditions of nutrition. Steiner (1934) details an

observation which seems to indicate that the males during their last moult do not take food and that shortage may induce the larvae of a given age to develop into males. *Anguillulina pratensis*, unlike *Heterodera marioni*, is probably able to move within the root during the whole of its larval period though the observations made during these experiments indicate that it makes little use of its power of motion. As *Tephrosia Vogelii* was used as the host plant in the experiments here described, it may not be legitimate to compare the ratio of males to females observed in them with that found in a population from large tea roots. In the tea roots the nematodes were certainly crowded at the perimeter of the lesions, so the high percentage of males there is associated with what may be termed overcrowded conditions. Crowding may result in adverse conditions of nutrition though food shortage may not be one of them. A shortage of suitable food is most likely to lead to the vagrant habit of this species observed by Steiner.

Sex ratio appears to influence the length of the pre-egg-laying period of adult females. It was to be expected that by the end of the 8th week of the experiments in series 3, nearly all females would have laid eggs some of which would have hatched, but Table 6 shows that only one of the nine females observed had laid eggs, eighteen eggs in all of which nine had hatched. This female was accompanied by a male.

The roots selected for examination at the 8th week were those which had been liable to infection by one larva only, from water, or by few larvae from sand. Forty-six roots were examined, but worms were found in only ten. Of these ten roots, seven contained solitary worms, two males and five females; one root contained three females, and another two females. The only root which contained eggs was the one already mentioned containing one male and one female. These observations indicate that *Anguillulina pratensis* is not normally parthenogenetic, and that unfertilized females do not lay eggs, at least for an abnormal period after becoming adult.

The results from earlier observations were examined to ascertain whether they would support such conclusions. At the end of the 5th week nine roots were observed to contain females only, and in only one of these were eggs also present. This root contained seven adults and sixty-one eggs. It is possible that one or more of these adults were males, but that fact was not recognized at the time owing to the position in which the worms were lying.

At the end of the 6th week four roots were observed to contain eggs but no males. One root contained seven adults and thirty-six eggs and the same comment may be made on this observation as was made on that at the end of the 5th week. The other roots contained one, two and three females with three, twelve and three eggs respectively. It is unlikely that mistakes were made concerning the sex of these adults, and certainly not of the solitary worm which had laid three eggs. These observations do not confirm entirely the conclusions drawn from the 8-week results, but it must be mentioned that although in the 5th and 6th weeks five roots were found to contain eggs but no male, there were also thirteen roots containing females only but with no eggs. The evidence on the whole indicates that in the absence of males, females do not lay eggs for at least 5 weeks after becoming adult as compared with about 15 days when males are present. These experiments offer no evidence to show whether in exceptional cases, when females lay eggs in the absence of males, such eggs develop normally.

*Egg-laying period.* The mean number of unhatched eggs decreased at each examination

of series 2 after the 3rd week. The eggs found at any time are those which have been laid in the previous 16 days or so, which is the mean period for egg hatching. The straight line which best fits the observed data of unhatched eggs, shown in Fig. 4, is represented by the equation $y = 27{\cdot}16 - 0{\cdot}55x$. When prolonged, this line cuts the $X$-axis at 49·4 days. If roots had been examined at that time practically no unhatched eggs, laid by the original females, would have been expected, though the new generation may have just started to lay. Egg laying by the original females would therefore have ceased about the 33rd day (i.e. 49·4 less an incubation period of 16·8 days).

This period is probably more nearly a maximum period of egg laying than a mean. If an individual maintained the mean rate of 1·68 eggs per day for 33 days, 55·4 eggs would be deposited; if the maximum rate of two per day were maintained, sixty-six eggs would be laid. Mention has already been made of one individual which at the end of 5 weeks had laid sixty-four eggs. This was an exceptional performance as the next best was forty-five. The contents of this root at the end of the 5th week were eleven adults (excluding the parent), thirty-seven larvae and sixteen eggs. It will be evident that the eggs from which the adults had developed must have been laid during the first 5 or 6 days after the female entered the root. It is possible therefore that this particular worm may have laid some eggs before it entered the roots. It is also probable that it had ceased egg laying before the end of the experiment, because the eggs from which the adults and larvae had developed must have been laid within the first 3 weeks, i.e. at an average rate of over two per day, whereas during the last 14 days only sixteen eggs had been laid. No other root at this examination contained so many unhatched eggs. This worm therefore laid regularly throughout the experimental period till a few days before its termination after 5 weeks. Other worms however did not begin to lay till the 3rd, 4th and even 5th week. We therefore regard 33 days or 5 weeks as an approximation of the maximum period of egg laying.

*Number of periods.* The number of eggs laid by *Anguillulina pratensis* as shown in these experiments is small compared with the numbers laid by other plant parasitic nematodes such as *Heterodera marioni*, and the question arises whether *Anguillulina pratensis* has but one egg-laying period or whether there is a succession with rest intervals between.

It has already been shown that young females normally begin to lay eggs about 15 days after becoming adult, but the absence of a male may cause a delay in egg laying. Amongst the roots in series 2 examined after 5 weeks were two which contained three and four unhatched eggs respectively, but contained no larvae or adults other than the original females with which the roots were inoculated. These eggs must have been laid within the previous 16 days otherwise they would have hatched. Owing to the small numbers the eggs were presumably laid a few days before the examination. In that case, these worms did not begin to lay until the 5th week after entering the root. As the worms were collected from a population with a high ratio of males to females it is improbable, but not impossible, that such worms were not fertilized.

The question arises therefore whether these worms were beginning to lay for the first time or whether they were beginning a second or third period after a rest of at least 1 month. It has already been shown that 92% of the worms found to be dead during the course of these experiments died without laying eggs. There are, therefore, two types of non-layers observed during the early weeks. The first consists of worms towards the end of their sexual life and which die without further egg laying. The second consists of worms which, either

because they were not fertilized or because they had not completed the necessary rest between egg-laying periods, did not begin to lay until the 4th or 5th week of the experiment. It will be noted that, if refertilization is necessary after a full period of laying, no worms were refertilized during the course of these experiments owing to the absence of males. The absence of refertilization, judging from results obtained in series 3, might prolong the rest (if any) between egg-laying periods.

If then there is a succession of egg-laying periods, the rest between such periods probably exceeds 3 weeks under the conditions of these experiments. One of the clearest indications of a succession of egg-laying periods would be roots in the 5th week containing a few adults, no larvae and a few eggs, so long as the adults other than the original one were not old enough to lay eggs for themselves. No such record was obtained.

There is therefore considerable doubt whether the females die a short time after completing an egg-laying period or whether, under favourable conditions, they may continue their sexual life. The data obtained from these experiments do not elucidate this point.

*Life cycle.* The life cycle, egg to egg, of *Anguillulina pratensis* in roots of *Tephrosia Vogelii* is completed in 45–48 days; which period may be divided into 15–17 days for the egg to hatch, 15–16 days as larval period and 15 days as adult before egg laying. This estimate is shorter than that (54–65 days) given by Hastings (1939) as the life cycle in oat seedlings.

## Summary

1. Measurements of *Anguillulina pratensis* collected from tea cortex and soils are summarized.

2. Attempts to maintain this species *in vitro* were not successful.

3. A rapid method for the determination of eelworm content of roots is described and its value in the study of the life cycle of *A. pratensis* is demonstrated.

4. Female *A. pratensis* lay, on the average, 1·6 eggs per day for a period rarely exceeding 5 weeks.

5. The eggs hatch in from 15 to 17 days and the larvae become adult in 15–16 days. The females begin to lay about 15 days later.

6. In the absence of males, egg laying is usually delayed.

We desire to thank Messrs H. T. P. Samarasekera and L. W. Perera for many of the measurements summarized in Table 1, and P. A. John for his patience in manipulating the hundreds of eelworms and their eggs used in these experiments.

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# SELECTIVITY IN BACTERIAL FOOD BY SOIL AMOEBAE IN PURE MIXED CULTURE AND IN STERILIZED SOIL

By B. N. SINGH

*Department of General Microbiology, Rothamsted Experimental Station, Harpenden, Herts*

(With Plate 4 and 3 Text-figures)

Earlier workers succeeded in getting "pure mixed cultures" of amoebae by feeding them on dead or living bacteria or yeasts. Oehler (1916, 1924*a*, *b*) found that amoebae eat more readily the Gram-negative species of bacteria than the Gram-positive species, and that of five species of amoebae some could grow on dead bacteria and yeasts, killed by heating, while others could grow only on living bacteria and yeasts. Severtzova (1928) studied the food relationships of soil amoebae with twenty-six species of soil bacteria, twelve moulds, four yeasts and two actinomycetes. She concluded: "If we examine the list of bacteria we find that neither the presence of proteolytic ferment, nor the ability of denitrification, of nitrogen fixation and of ammonification, nor the capacity of motion, nor the presence of pigmentation and, certainly, not the relation to Gram staining can account for the selective action by the amoebae. The small, motile, non-spore-bearing bacteria as well as the small immotile cocci seem to represent the most suitable food the amoebae can find in the soil." Among the edible species of bacteria some were more readily accepted by amoebae than others. Among the spore-forming bacilli the vegetative forms were preferred to the spores.

Cutler & Crump (1927, 1935), using *Hartmanella hyalina*, found that different species of bacteria appear to have different nutritive values. The bacterial species "YB" and "SE" are of the same size and shape, yet their nutritive value, judging by the rate of reproduction of the amoebae feeding on them, is quite different. They concluded that this is a true feeding effect because they did not observe that the waste products of the bacteria had a bad effect on the amoebic growth. Rice (1935, 1938), using *Flabellula mira* and three other species of marine amoebae, observed that whether the bacteria were Gram-positive or Gram-negative made little difference to their utilization as food by the amoebae. Of ten species of bacteria, *Serratia ruber* was untouched by the amoebae; some of the species were eaten by all the amoebae, while others were eaten by some species of amoebae and were untouched by others.

## A. Selectivity in bacterial food by soil amoebae in "pure mixed" culture

### *Material and methods*

A small and a larger species of soil amoebae belonging to the Limax group were obtained from Barnfield farmyard manured soil (Rothamsted Experimental Station, Harpenden). A fragment of soil was placed in the centre of a bacterial circle on an agar plate and incubated at 20° C. When, within a few days, the amoebae had moved outside the bacterial circle, some of them were put again into the centre of a freshly prepared bacterial circle. This process was repeated till a pure amoebic culture was obtained. To get a pure line culture from a single amoeba, it was washed several times in

sterilized saline and then placed in the centre of a small bacterial circle of the given species as food. Micropipettes were used for isolating the amoebae and transferring them from one washing solution to the next.

### *A method for studying food selection by amoebae*

Amoebae migrate in all directions on the plate when they are placed either in the centre of bacterial circles or stars. This difficulty was overcome as follows: pieces of hard glass tube are pulled into thin tubes of uniform size, broken into small pieces, and arranged inside Petri dishes in as many radii as desired (Text-fig. 1 and Pl. 4). The Petri dishes with the tubes are sterilized and agar is poured between the tubes with a fine pipette. The tubes are arranged with small sterile forceps, and stick to their positions when the agar has solidified. Bacterial streaks are made on the agar and amoebae are placed in the centre of them.

Throughout the experiments 2% agar containing 5 g./l. NaCl was used, this giving better results than nutrient agar because the bacteria are not able to multiply as quickly. The species of bacteria used and the sources from which they were obtained are given in Table 1. In all the feeding experiments bacterial cultures of 3–10 days were used. The temperature of incubation was 20° C. For a comparative study of the preference in food, bacterial cultures of the same age were always used.

### *Observations*

#### *Species of* Aerobacter *preferred by amoebae.*

Five strains of *Aerobacter* were used (1912, 08, 07, 1734 and 2006). They show identical morphology, and more or less similar physiological reactions (Table 1). A series of plates was made by the method described and amoebae were inoculated in the centre of the bacterial stars. Amoebae do not show extreme difference in their preference towards the *Aerobacters* but they destroy some strains in larger numbers than others, and move along the bacterial radii in those cases much more quickly. Species 1912 is the food preferred most by the amoebae, and the others are accepted in the following order 08, 07, 1734 and 2006 (Pl. 4, fig. 1).

#### *Various degrees of selectivity in bacterial food as exhibited by the soil amoebae.*

The seventeen kinds of bacteria used (Table 1) may be grouped according to their suitability as food for the amoebae: λT 20, 1912, 08, 07, 1734, 2006 and S 21 are eaten readily by the amoebae, N 16(i) and 4045 are eaten slowly, and 0312, 0746, 2881, 5654, 5431, 4022, 4031 and R are either untouched, or are eaten very slightly if they are the only food available.

In selectivity experiments bacteria were inoculated along the radii, each radius consisted of a different kind of bacterium, and the amoebae were inoculated in the centre. The preference for one type of food as opposed to another was judged by the amount of bacteria destroyed along each radius in a given time. All the experiments were repeated several times before final conclusions were arrived at. In each experiment both edible and non-edible bacteria were used. Among the readily accepted food types the amoebae eat most readily species λT 20 and move along this bacterial radius much more quickly than along the others in a given time. The other types of bacteria are preferred by the amoebae in the following order, 1912, S 21, 08, 07, 1734 and 2006. The choice made by the amoebae among the bacteria 4045, N 16(i), R, 4022, 4031, 5431, 5654, 0312, 0746 and 2881 is as follows: 2881, 0312, 0746, 5654, 5431 are never touched even when they are the only available food; R, 4022 and 4031 are on rare occasions very slightly eaten by the small amoeba used in these experiments but not by the larger type; 4045 and N 16(i) are slowly

TABLE 1. *Sources and characters of bacteria used in experiments*

| Strain | Motility | Morphology | Liquefaction | Gelatine | Milk | Dextrose | Laevulose | Sucrose | Lactose | Dulcitol | Nitrate reduction | Indol | Locality | Colour | Gram staining |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 07 | ◘ | Short rod | o | Thread | Acid curd R.L. | G 4·0 | G 4·6 | G <4·0 | G <4·0 | 6·9 | +++ | ++ | Milk waste | Milky white | − |
| 08 | + | Short rod | o | Thread | Acid slow curd R.L. | G 5·8 | G 5·5 | G 5·5 | G 5·3 | 6·9 | +++ | o | Milk waste | Milky white | − |
| 1734 | o | Short rod | o | Thread | Acid curd R.L. | G 4·7 | G 4·3 | G 4·2 | G 4·4 | 6·9 | +++ | Trace | Milk waste | Milky white | − |
| 1912 | o | Short rod | o | Thread | Acid curd R.L. | G 4·7 | G 4·7 | G 4·6 | G 4·1 | 6·9 | +++ | o | Milk waste | Milky white | − |
| 2006 | + | Short rod | o | Thread | Acid curd R.L. | G 5·6 | G 5·9 | G 4·3 | G <4·0 | G 4·4 | +++ | o | Milk waste | Milky white | − |
| S 21 | o | Very small rod | + | Cup | R.L. | 6·5 | 6·3 | 7·1 | 6·9 | 7·0 | +++ | Trace | Barnfield F.Y.M. | Milky white | − |
| N 16(i) | o | Sarcina | o | Thread | o | 6·9 | 6·9 | 6·9 | 6·9 | 7·0 | o | o | Sugar effluent | Citron yellow | + |
| λT 20 | + | Cocci | + | Saucer | o | 6·9 | 6·9 | 6·9 | 6·9 | 7·0 | o | o | Sugar effluent | Yellow ochre | + |
| 2881 | + | Very short rod | + | Infundibular | Acid curd | <4·0 | <4·0 | <4·0 | 6·6 | 6·7 | +++ | + | Milk waste | Eugenia red | − |
| 0312 | + | Short to medium rod, pairs: short chains | o | Thread | (Alk.) | 6·6 | . | . | 6·6 | 7·0 | o | o | Milk waste | Tawny | − |
| 0746 | o | Thin rod | o | Thread | Alk. | 6·7 | 7·1 | 6·9 | 6·9 | 7·0 | +++ | o | Milk waste | Flesh ochre | − |
| 4022 | o | Very small rod | o | Thread | R.L. | 5·2 | 5·5 | 5·4 | 6·6 | 7·0 | + | o | Barnfield F.Y.M. | Citron yellow | − |
| 4031 | o | Medium rod: single and pairs | o | Thread | o | 6·7 | 6·4 | 6·9 | 6·7 | 7·0 | o | o | Barnfield F.Y.M. | Antimony yellow | + |
| 4045 | o | Sarcina | + | Saccate | o | 6·8 | 6·9 | 6·9 | 6·9 | 7·0 | o | o | Broadbalk Plot 3 | Citron yellow | + |
| 5431 | + | Medium rod | o | Thread | Pept. | 6·3 | 5·5 | 5·7 | 6·6 | 7·0 | +++ | o | Barnfield F.Y.M. | Blackish violet | − |
| 5654 | o | Small oval rod | + | Infundibular | Curd turns pink | G 4·2 | G 4·5 | G <4·0 | 6·0 | 7·0 | +++ | o | Park Grass Plot 2 | Spinel red | − |
| R (Radiobacter) (N.C.T.C. 1376) | + | Small rod | o | Thread | (Alk.) | 6·5 | 6·3 | 6·3 | 6·7 | 6·8 | +++ | o | Soil | Milky white | − |

eaten, but the amoebae prefer 4045 to N 16(i). It has been found that both species of amoebae prefer the same kind of food, to the same degree, except in the case of bacteria R, 4022 and 4031.

When the amoebae are inoculated in the middle of bacterial stars consisting of both edible and non-edible bacteria, they move in large numbers in all directions in search of food. After reaching the suitable food supply they eat, multiply and move along the bacterial stars. When they reach the non-edible food supply, they either encyst within a short time or move along these radii to some distance without destroying the bacteria, and finally encyst. It is possible also that some of them die.

*Selectivity of food by soil amoebae when two kinds of bacteria are arranged side by side.*

Two streaks, one of edible and the other of non-edible bacteria, were made touching each other (Pl. 4, figs. 3, 4): the amoebae were inoculated in the centre of the bacterial streaks. The amoebae eat the edible type of bacteria and leave the non-edible ones. Pl. 4, fig. 4 shows that bacteria 4031 is also slightly eaten by the amoebae. In the presence of species 2881 (*B. prodigiosus*), however, the amoebae are able to eat the readily edible species S 21 only slightly (Pl. 4, fig. 3). In some cases it happens that, in the presence of *B. prodigiosus*, amoebae eat a little edible food for a day or two and then encyst. Pl. 4, figs. 3, 4 show clearly that the amoebae do not destroy the bacterial species 4022, 0312, 5654, 2881, R, 5431 and 0746 even when they are present side by side with edible types and touching each other. The cause of the difference between 2881 and the other non-edible species will be discussed later.

*Selectivity of food by amoebae among different kinds of edible and non-edible types of food supply.*

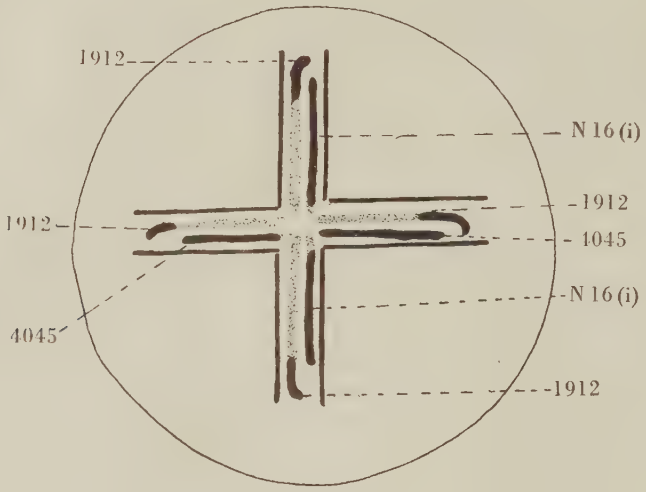
Bacteria were arranged as shown in Pl. 4, figs. 2, 5 and 6, and amoebae were inoculated in the centre and allowed to move in all directions: the photographs show that the amoebae although moving in masses in between the non-edible food supply, ate only the edible food and left the bacterial species 5431, 4022, 0746, R, 2881 and 5654.

Amoebae either encyst when they reach unfavourable food, or they move on in search of a favourable food supply when they feed and multiply. Pl. 4, fig. 5 was taken 5 days after inoculating with amoebae: some of the edible bacteria are either untouched by the amoebae or eaten only very slightly. Pl. 4, fig. 6 was taken 8 days after the inoculation of the amoebae: in this the amoebae have eaten practically all the edible kinds of bacteria, and have left the non-edible kinds.

*Preference in food by amoebae among readily accepted and non-readily accepted food supply.*

Readily accepted and less readily accepted bacteria were arranged as shown in Text-fig. 1 and amoebae were placed in the centre touching the bacteria 1912. In most cases the amoebae ate first the readily accepted food 1912, and when this food was finished the amoebae began to eat the less readily accepted food, 4045 and N 16(i). Sometimes the amoebae ate very slightly bacteria N 16(i) and 4045 even when there was plenty of readily accepted food supply. If amoebae are cultured on bacteria 4045 or on N 16(i) for some time and then inoculated in the centre of the bacterial streaks as shown in Text-fig. 1, they

start eating both the bacteria (4045 and 1912) at the same time. That is, if amoebae are accustomed to eat less readily accepted bacteria they do not show the preference for eating the most readily accepted food first and later the less readily accepted ones as described above.




Text-fig. 1.

*Why are some bacteria readily accepted, other less readily, and the rest untouched by the amoebae?*

To test the presence of exo-toxins among the non-edible bacteria, they were plated on agar slopes and allowed to grow thickly for 10–15 days. The bacteria were then scraped off and the agar was melted and poured into sterile Petri dishes. In each Petri dish containing the exo-toxin of one kind of bacteria several bacterial circles of edible bacteria were made, and amoebae were inoculated in the centre. After a few days the amoebae ate the food supplied to them, and destroyed the edible kinds of bacteria in large numbers. The same result was obtained, when the two types of bacteria were put together touching each other. These two experiments clearly show that the non-edible types of bacteria used (except 2881) do not produce exo-toxin in sufficient quantity to prevent the amoebae from eating the edible food supply. In the presence of the exo-toxin of bacteria 2881 the amoebae are either unable to eat the edible food supply or they eat it very slightly and finally encyst or die. This is the case whether the agar containing the exo-toxin is used or the bacteria 2881 is put side by side with an edible type of bacteria, and touching it.

To test the presence of endo-toxins in non-edible bacteria, they were crushed in a glass bacterial mill with normal salt solution, filtered through an L3 candle, and the filtrates were collected. A series of bacterial circles consisting of edible kinds of bacteria was made on agar plates, and one or two drops of liquid containing the products of the crushed bacteria were added to each of the bacterial circles: the amoebae were inoculated in the centre. After 2–3 days the amoebae had destroyed the edible bacteria in very large numbers, showing that the non-edible bacteria did not contain endo-toxin. Sometimes it is difficult to filter proteins through bacterial candles, and it may be possible that endo-toxin of the

bacteria does not reach the filtrate. To test this point the non-filtered crushed bacteria were used in the above experiments: the result was the same. It may be possible that the quantity of endo-toxin is so little as to have no effect on the amoebae.

It is interesting to note that the crushed product or the crushed and filtered product of bacteria 2881 (*B. prodigiosus*) has no effect in preventing amoebae from eating edible food.

It is clear from Table 1 that these amoebae have no special preference for the Gram-negative or the Gram-positive bacteria. The bacteria 07, 08, 1734, 1912, 2006 and S21 are Gram-negative and are readily eaten by amoebae while 2881, 0312, 0746, 4022, 5431, 5654 and R which also are Gram-negative are not touched by the amoebae. The same is true for Gram-positive bacteria, i.e. some of them are eaten by the amoebae while others are not. A large number of Gram-positive bacteria were not used, but it seems certain that, contrary to the claims of Oehler (1916, 1924 *a*, *b*), the amoebae have no special preference for Gram-negative bacteria.

The majority of the bacteria used were either short, very small, or medium-sized rods (Table 1). Amoebae eat some of these bacteria and leave the others, showing that the size of the bacteria has no evident relationship with edibility. The same is true in the case of motile and non-motile bacteria. Pigmentation in bacteria does not seem to be related to their edibility; of the pigmented bacteria, shown in Table 1, λT 20 is eaten readily by the amoebae, N 16(i) and 4045 are eaten slowly, and the rest are either very slightly eaten sometimes (4022, 4031) or completely untouched (5654, 5431, 0746, 0312, 2881).

## B. Selectivity in bacterial food by soil amoebae in sterilized soil

### *Methods*

Soil from the plot in Barnfield, annually manured with 14 tons of farmyard manure per acre, was selected as experimental material. It is a heavy clay soil with a *p*H from 7·1 to 7·3. The soil was air-dried, powdered and sieved. Tubes containing 20 g. of soil were autoclaved at 15 lb. pressure for 1 hr. on three successive days. Tests showed that this method sterilized the soil with very little change in the *p*H value. 300 g. portions of soil were placed in large sterile Petri dishes. The inocula used were:

(1) Bacterial species 5654 alone.
(2) Bacterial species 5654 + bacterial species 4045.
(3) Bacterial species 4045 alone.
(4) Bacterial species 4045 + bacterial species 5654 + one species of soil amoeba.

The amoebae were cultured on species 4045 for 4–6 months, and tests showed that in the culture of amoebae only that species was present. In a throat spray fitted with the finest nozzle obtainable the suspensions of bacteria and amoebae were made in saline. In one spray there was the suspension of species 4045; in a second, of species 5654; and in a third, of amoebae grown on species 4045. The amoebae were mostly in the cystic condition, and there were very few bacteria (4045) in the suspension containing the cysts.

Sterile tap water was sprayed on the soil contained in the large Petri dishes, followed by the sprays of bacteria and amoebae. By this method it is not possible to inoculate the bacteria into the soils in equal numbers, but care was taken to ensure that approximately the same numbers of bacteria were inoculated in the different Petri dishes containing the soils. The water content of the soil was 25%. Every 3–4 days sterile tap water was sprayed on to the soils to bring their moisture content to 25%. The Petri dishes were kept in an incubator at room temperature. The numbers of bacteria were counted on the next day after inoculating them in the soil and further counts were taken as shown in Table 2. In the beginning of the experiment it was not possible to count the numbers of bacteria every day owing to the disturbance created by the outbreak of the war.

To count the numbers of bacteria 10 g. of soil was taken from each dish, diluted to 1/500,000 in sterile saline and plated out: from each of the final dilutions five plates were poured, and the number

TABLE 2. *The numbers of bacteria are given in millions/g. of soil*

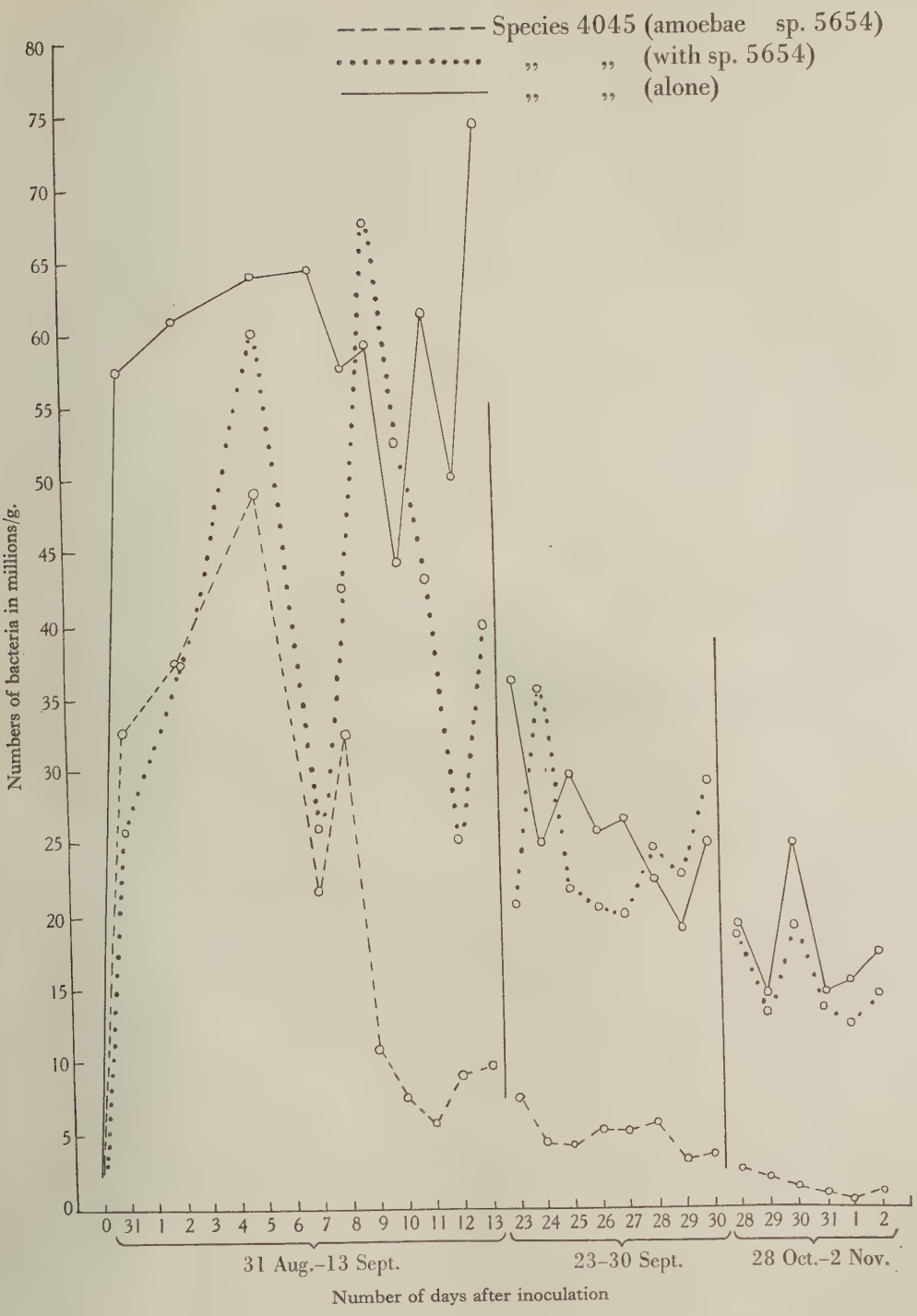
| Date | A bacteria + amoebae | | B control bacteria only | | C control bacteria only | D control bacteria only |
|---|---|---|---|---|---|---|
| 1939 | 5654 | 4045 | 5654 | 4045 | 5654 | 4045 |
| 31 Aug. | 231·5 | 32·5 | 204·5 | 26·0 | 239·0 | 57·5 |
| 2 Sept. | 170·0 | 37·5 | 187·5 | 37·5 | 253·7 | 61·2 |
| 5 ,, | 186·2 | 48·7 | 275·0 | 60·0 | 256·2 | 63·7 |
| 7 ,, | 97·2 | 22·0 | 172·5 | 26·2 | 240·0 | 64·0 |
| 8 ,, | 70·7 | 32·5 | 121·7 | 42·5 | 122·2 | 57·5 |
| 9 ,, | 52·2 | 10·5 | 107·5 | 67·5 | 176·2 | 58·7 |
| 10 ,, | 28·0 | 7·5 | 58·2 | 52·5 | 87·5 | 43·7 |
| 11 ,, | 75·0 | 6·2 | 119·7 | 43·2 | 227·0 | 61·5 |
| 12 ,, | 54·7 | 9·0 | 92·2 | 25·2 | 139·0 | 50·2 |
| 13 ,, | 118·2 | 9·5 | 176·7 | 40·0 | 162·5 | 74·0 |
| 23 ,, | 95·5 | 7·5 | 112·0 | 21·5 | 109·0 | 36·5 |
| 24 ,, | 86·5 | 4·5 | 118·5 | 36·0 | 105·5 | 25·0 |
| 25 ,, | 91·2 | 4·2 | 115·0 | 22·0 | 142·0 | 29·5 |
| 26 ,, | 67·5 | 5·5 | 107·5 | 21·0 | 152·5 | 26·0 |
| 27 ,, | 57·2 | 5·5 | 116·0 | 20·0 | 97·0 | 27·0 |
| 28 ,, | 43·0 | 6·5 | 81·5 | 24·5 | 100·0 | 22·5 |
| 29 ,, | 43·2 | 3·2 | 86·0 | 23·0 | 80·0 | 19·0 |
| 30 ,, | 95·0 | 3·7 | 99·0 | 29·0 | 72·5 | 25·0 |
| 28 Oct. | 98·0 | 2·5 | 92·5 | 18·5 | 114·5 | 19·0 |
| 29 ,, | 90·5 | 2·0 | 86·5 | 13·5 | 94·0 | 14·0 |
| 30 ,, | 99·5 | 1·6 | 116·0 | 19·5 | 145·0 | 25·0 |
| 31 ,, | 93·5 | 1·0 | 62·0 | 13·5 | 76·0 | 14·5 |
| 1 Nov. | 62·0 | 0·8 | 56·0 | 12·5 | 76·5 | 15·5 |
| 2 ,, | 78·0 | 1·1 | 75·5 | 14·5 | 73·0 | 17·5 |

of bacteria in 1 g. of the soil was calculated. The Petri dishes were incubated for 2–3 weeks at room temperature. When the number of bacteria 4045 became low in the soil, five extra plates were poured from the dilution of 1/50,000 to count the number of that bacteria. The characteristics of the two types of bacteria used are given in Table 1. The numbers of both the types of bacteria were found by counting the two types of coloured colonies developing on the agar plates. Bacteria 4045 produced citron yellow colonies, but the spinel red bacteria (5654) sometimes produced white colonies as well. The red and the white colonies produced by the bacteria 5654 are of similar shape on agar plates. The number of active and cystic amoebae was counted only a few times during the course of this experiment by Cutler's method (1920).

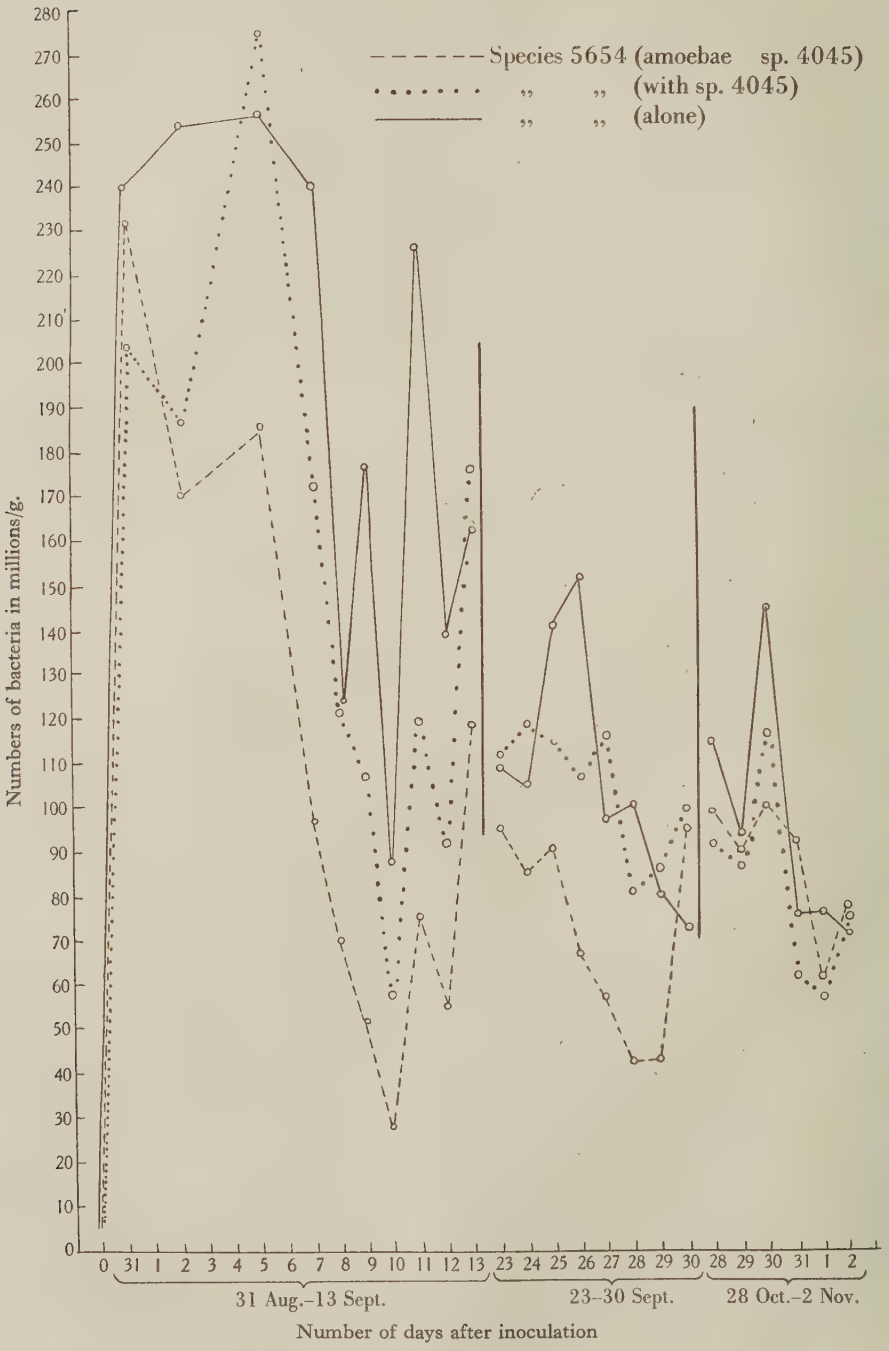
Before selecting the species 4045 and 5654 preliminary tests were made with several species of soil bacteria to find out if there was antagonism between them: presence of species 4045 does not stop the growth of species 5654 and conversely.

## EXPERIMENTAL RESULTS

As pointed out in § A, bacteria 4045 is slowly eaten by the amoebae, and bacteria 5654 is not touched by them in pure culture. The present work was carried out to ascertain if similar results are obtained in sterilized soil. Results are shown in Table 2 and Text-figs. 2 and 3. In the case of bacterial species 4045 the presence of amoebae does not influence the result for the first week. The fluctuations in bacterial numbers, both in the soil containing the amoebae as well as the controls, are more or less similar. This can be explained by the fact that amoebae were inoculated mostly as cysts, and it takes some time before their active number is large enough to destroy the bacteria in sufficient numbers. A similar result was obtained by Cutler (1923). The results obtained from 9 to 13 Sept. show clearly that amoebae are definitely exerting their phagocytic influence on bacteria 4045. The


Species 4045 (amoebae sp. 5654)
,, ,, (with sp. 5654)
,, ,, (alone)
Numbers of bacteria in millions/g.
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
0 31 1 2 3 4 5 6 7 8 9 10 11 12 13 23 24 25 26 27 28 29 30 28 29 30 31 1 2
31 Aug.–13 Sept.
23–30 Sept.
28 Oct.–2 Nov.
Number of days after inoculation

Text-fig. 2.


Species 5654 (amoebae sp. 4045)
,, ,, (with sp. 4045)
,, ,, (alone)
Numbers of bacteria in millions/g.
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280
0 31 1 2 3 4 5 6 7 8 9 10 11 12 13 23 24 25 26 27 28 29 30 28 29 30 31 1 2
31 Aug.–13 Sept.
23–30 Sept.
28 Oct.–2 Nov.
Number of days after inoculation

Text-fig. 3.

count from 23 to 30 Sept. shows that, in the soil in which the amoebae were present, the number of bacteria 4045 is gradually falling and there is practically no sign of their increment or fluctuation. The bacterial numbers in control experiments are more or less stationary, with slight fluctuations (compare Cutler *et al.* 1922, and Thornton & Gray, 1934). The result obtained from 28 Oct. to 2 Nov. is more or less the same as that obtained from 23 to 30 Sept. This experiment clearly shows that amoebae destroy the bacteria 4045 in sterilized soil, the number of bacteria 4045 being at a much lower level in the soil containing amoebae than in the control soils (Table 2 and Text-fig. 2).

The results obtained in the case of bacteria 5654 are shown in Table 2 and Text-fig. 3. The counts from 7 to 12 Sept. show that the amoebae reduce the number of this bacteria. In pure culture bacteria 5654 is never eaten by the amoebae, but it seems that amoebae are able to eat bacteria 5654 up to the counting period of 30 Sept. The most interesting part of the result in the case of bacteria 5654 is shown by the bacterial counts from 28 Oct. to 2 Nov., during which period the amoebae are not able to eat the bacteria 5654. When the protozoan count was taken on 30 Oct. the amoebae were mostly found to be in the cystic condition (Table 3). During the period 28 Oct.–2 Nov., the number of bacteria 4045 is very low, and although the number of bacteria 5654 is high yet the amoebae are found in the cystic condition. It is reasonable to draw the conclusion that amoebae are unable to eat bacteria 5654 from 28 Oct. to 2 Nov. when the bacterial food 4045 was almost exhausted, and so they encyst. It is interesting to note that although there is plenty of food for the amoebae, mostly consisting of bacteria 5654, yet the amoebae do not eat that food, a result in complete agreement with that obtained in pure culture experiments.

The numbers of active and cystic amoebae were counted only three times during the experiment (Table 3) and it is clear that amoebae were present both in the active and cystic condition in the soil and that their number increased during the experiment. When the last count was taken, the majority were found to be in the cystic condition.

Table 3

| Date | Amoebae (active + cystic) | Amoebae (cystic) | Amoebae (active) |
|---|---|---|---|
| 12 Sept. | 118,000 | 46,000 | 72,000 |
| 26 Sept. | 220,000 | 118,000 | 102,000 |
| 30 Oct. | 220,000 | 220,000 | — |

By comparing the results in control experiments B, C and D (Table 2) it is clear that the two types of bacteria (5654 and 4045) selected for the present work do not arrest the growth of each other. The fluctuations in their numbers in C and D are more or less the same as in B, in which there are both the types of bacteria 5654 and 4045.

## Discussion

It has been the experience of all previous workers that the main food of amoebae consists of bacteria either living or dead. Amoebae have also been cultivated on yeasts, and they may feed on fungal spores, algae, etc. Even, however, in the case of dead bacteria, killed either by heating or by any other way, there are only a few cases where amoebae have been cultured successfully. So far it has not been possible to cultivate amoebae on mainly liquid media without particulate food as is possible in the case of saprophytic or autotrophic

protozoa. Cutler, Crump and others believe that amoebae may be one of the factors keeping the number of bacteria in check in the soil, and Cutler *et al.* (1922) observed a definite inverse relationship between bacterial numbers and active amoebae in field soil. In view of the possibility that amoebae and probably also the flagellates lead a holozoic existence in the soil, where there is plenty of bacterial food supply, it is desirable to know whether amoebae eat all types of bacteria or whether they select among them, and in the latter case to find the reason for the selectivity.

Among the Gram-positive and Gram-negative bacteria tested amoebae showed no special preference for Gram-negative. This is contrary to the findings of Oehler (1916, 1924 *a*, *b*), but agrees with the results of Severtzova (1928) and Rice (1935). Bacterium λT 20 is Gram-positive yet it is the best kind of food for the amoebae. Pigmentation in bacteria has no relation to their edible or non-edible quality. Similarly, in most cases, there is no evidence of the presence of endo- or exo-toxin in the non-edible bacteria which prevents them from being eaten by the amoebae, although the production of exo-toxin was observed in bacteria 2881 and may prevent it from being eaten by amoebae.

Among seventeen different kinds of bacteria, it has not been possible to discover any particular character which prevents them from being edible or non-edible for the amoebae. It may only be said that some bacteria are preferred by the amoebae to the others; e.g. some Aerobacters more than others. By developing a technique for carrying out feeding experiments with amoebae, it has been possible to show more clearly that amoebae are able to select their food, amoebae first eating the readily acceptable food and later the slowly acceptable food.

By inoculation experiments carried out in sterilized soil, it has been shown that amoebae are able to select their food, although the result is not exactly the same as in pure culture experiments, and the edible bacteria are not destroyed in large numbers. Severtzova (1928, p. 177) says: "Excepting one case the presence of amoebae in the soil did by no means affect the normal development of the bacteria in it. The amoebae did not hinder the development of the bacteria, even in those cases where the multiplying amoebae were placed under exceptionally favourable conditions. Concerning the question, whether amoebae can as sharply manifest their selective ability in the soil, as they do on artificial media, our preliminary experiments have given an affirmative answer in one point; in the presence of amoebae spore-bearing bacilli developed in the soil far more abundantly than did small, motile, non-spore-bearing bacteria, perhaps, because the latter were more readily attacked by the amoebae, though other causes may be responsible." In the absence of the experimental data, it is difficult to comment on the conclusions drawn by Severtzova. It must be pointed out that the amoebae are able to select edible from non-edible food in sterilized soil, although the numbers of bacteria destroyed by the amoebae in sterilized soil is very small compared with those in pure culture experiments. Further, Cutler (1923) showed that the presence of active amoebae in sterilized soil keeps the numbers of bacteria below the level that they would otherwise have attained.

It may be pointed out that species 4045, which has been selected in the present experiment, is not among those which are readily eaten by the amoebae in pure culture. It seems likely that, if instead of species 4045, a bacteria which is readily eaten by the amoebae had been selected it would have given much better results in selectivity experiments than has been obtained with bacteria 4045.

## Summary

1. A plate culture method, for carrying out work on the selection of bacteria for food by amoebae, is described.

2. It is shown that some species of *Aerobacter* are preferred by amoebae to others, though they are all morphologically and more or less physiologically identical.

3. Of a number of bacterial species, mostly rods of different sizes, some were completely rejected as food by the amoebae. Among those that were selected as edible, the amoebae showed varying degrees of preference. It has not been possible to determine why amoebae eat some kinds of bacteria rather than others, except possibly in the case of bacterium 2881.

4. Amoebae have no special preference for Gram-negative bacteria in comparison to the Gram-positive ones.

5. Amoebae are able to select edible from non-edible food whether the two types of food are offered side by side or on opposite sides of the plate culture.

6. It has been shown that the amoebae are able to select their food in sterilized soil, among edible and non-edible species of bacteria. In sterilized soil the amoebae do not destroy edible bacteria in large numbers as is seen in pure culture experiments.

7. The number of edible bacteria is very much reduced when amoebae are present. It seems very likely that the amoebae are not able to keep the numbers of non-edible bacteria in check as is the case with edible types of bacteria.


My best thanks are due to Mr D. Ward Cutler and Miss L. M. Crump for their never failing interest, suggestions and helpful criticisms throughout the course of this work, and for giving me the different species of bacteria from their personal collections.

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## EXPLANATION OF PLATE 4

Fig. 1. Preference in the bacterial food, consisting of Aerobacters, by soil amoebae. 3 days old culture of amoebae.

Figs. 3, 4. Amoebae eat the edible and leave the non-edible kinds of bacteria when they are put side by side and touching each other. 4 days old culture of amoebae.

Figs. 2, 5 and 6. Amoebae select the edible from the non-edible food when several types of food are present. Fig. 5 is 5 days old, Fig. 6 is 8 days old, Fig. 2 is 6 days old culture of amoebae.

(*Received 27 July* 1940)

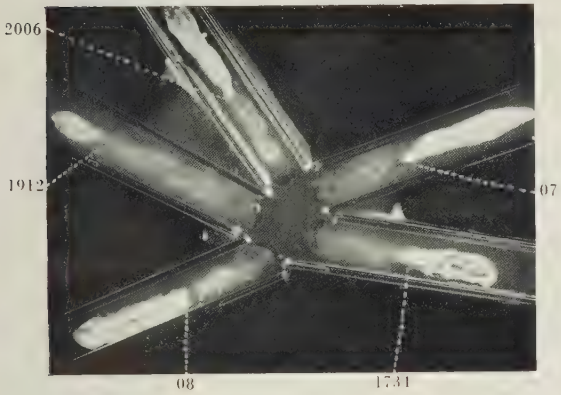


Fig. 1.

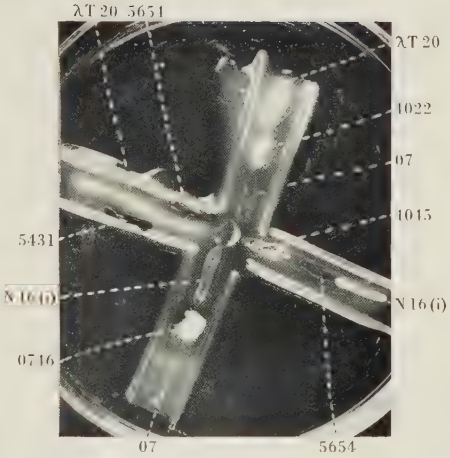


Fig. 2.

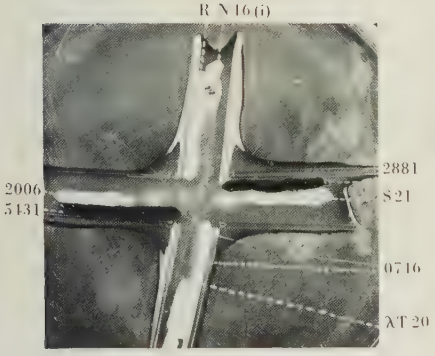


Fig. 3.

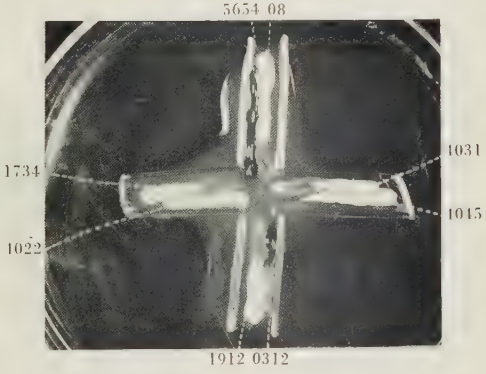


Fig. 4.

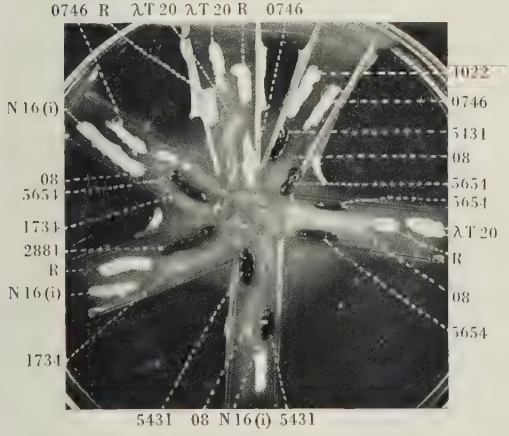


Fig. 5.

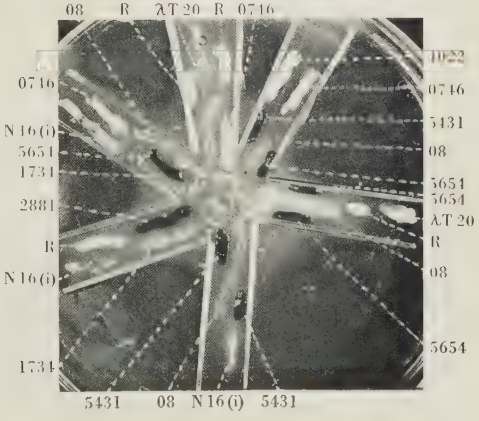


Fig. 6.

SINGH.—Selectivity in Bacterial Food by Soil Amoebae in Pure Mixed Culture and in Sterilized Soil (pp. 52–64)

# THE INFLUENCE OF DIFFERENT BACTERIAL FOOD SUPPLIES ON THE RATE OF REPRODUCTION IN *COLPODA STEINII*, AND THE FACTORS INFLUENCING ENCYSTATION

By B. N. SINGH

*Department of General Microbiology, Rothamsted Experimental Station, Harpenden, Herts*

Various workers have claimed that factors such as drying of the culture medium, metabolic products of bacteria and Protozoa, age of the culture, hydrogen-ion concentration, lack of oxygen, physiological periodicity, lack or abundance of food, crowding of the individuals, temperature, etc. influence encystation in different Protozoa (see Penn, 1935, for bibliography). A knowledge of the causes of cyst formation might throw light on the role played by the Protozoa in soil economy, and especially on the cause of the daily fluctuations in the number of Protozoa recorded by Cutler *et al.* (1922). It was desired to find out whether the rate of reproduction is accelerated, in the case of *Colpoda*,[1] by the quality of the bacterial food or whether it is due to an "X" substance as suggested by Robertson (1921*a*, *b*, 1924*a*, *b*, *c*, 1927). In this connexion a strain of a nodule bacterium (310)[2] has been used: this strain produces growth-promoting substance for plants.

## Material and methods

*Colpoda steinii*, a common soil form, was obtained by diluting Barnfield soil (Rothamsted Experimental Station) in sterilized hay infusion of 1·5 % strength, and incubating at 19–20° C. for a few days. The animals used were originally derived from a single individual. Individuals were isolated with fine glass tubing and washed at least five times in sterile soil extract until free from other micro-organisms (see Parpart, 1928). The washed individuals were placed in hollow-ground slides in two or three drops of soil extract, and were supplied with a known species of bacteria as food. The slides were kept in Petri dishes as moist chambers and incubated for 2–3 days at a temperature of 19–20° C. till a large number of animals were obtained. Aseptic conditions were maintained throughout.

For counting the organisms haemocytometers with the Cropper ruling were used. The rate of reproduction for any time is calculated by the formula (log *B*–log *A*)/log 2, where *B* is the number at the end and *A* the number in the beginning. Lugol was a satisfactory means of killing the animals and they did not burst after death. Sometimes there is considerable difference in the number of individuals counted when several consequent counts are taken. To reduce this error the animals in all the 625 squares of the Cropper ruling was counted, and the average of four such counts was taken as the number of individuals present at each counting period. The number of bacteria at each counting period was counted in a Thoma haemocytometer. The animals grow well in hay infusion, but after some time the solution becomes wholly unsuitable for the uniform suspension of the organisms. Soil extract was used throughout as a culture solution. The initial reaction of the medium was approximately *p*H 7·0.

[1] This genus was most probably used by Robertson in his later experiments, although he names his genus *Colpidium*. As far as the writer is aware the reproduction in *Colpidium* always takes place by binary fission as happens in *Paramoecium* and other ciliates. It is only in *Colpoda* that the individuals round up and form cysts before they divide into four or eight individuals. Therefore, I think that Robertson used in his experiments *Colpoda* and not *Colpidium*.

[2] Given to me by Dr H. Nicol of the Bacteriology Department, to whom I wish to convey my sincere thanks.

## Observations

### (*a*) *The rate of reproduction in* Colpoda steinii *when fed with different bacteria*

Five types of bacteria were selected (1734, 07, R, S 21 and 310): species 1734 and 07 belong to the *Aerobacter* group and are more or less physiologically identical, R is a Radiobacter, 310 is a strain of nodule bacteria, and S 21 is a soil form (Table 1).

Table 1. *Characters of bacteria used as food*

| Strain | Motility | Morphology | Liquefaction | Gelatine | Milk | Dextrose | Laevulose | Sucrose | Lactose | Dulcitol | Nitrate reduction | Indol | Locality | Colour | Gram-stain |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | G | G | G | G | | | | | | |
| 07 | o | Short rod | o | Thread | Acid curd R.L. | 4·0 | 4·6 | <4·0 | <4·0 | 6·9 | +++ | ++ | Milk waste | Milky white | − |
| | | | | | | G | G | G | G | | | | | | |
| 1734 | o | Short rod | o | Thread | Acid curd R.L. | 4·7 | 4·3 | 4·2 | 4·4 | 6·9 | +++ | Trace | Milk waste | Milky white | − |
| S 21 | o | Very small rod | + | Cup | R.L. | 6·5 | 6·3 | 7·1 | 6·9 | | +++ | Trace | Barnfield F.Y.M. | Milky white | − |
| R (Acromobacter radiobacter) | + | Small rod | o | | | Acid | Acid | Acid | Neutral | Neutral | +++ | | Soil | Milky white | − |

In a subculture made from a 48 hr. old parent culture, there is a lag period of shorter duration compared with the subcultures made from 72 or 96 hr. old parent. Cutler & Crump (1923*a*, *b*, 1924) found the same thing in the case of *Colpidium colpoda* and *Oicomonas termo*. In subcultures of 4 or 5 days old parent, the death rate is heavy during the first period of 24 hr., but the individuals that survive are able to reproduce more vigorously, and their reproductive rates, after the lag period, are generally higher than subcultures made from 48 hr. old parents. The lag period is due to the death of the individuals after inoculation into a fresh medium, and no resistant cysts have been seen to be formed during this period.

Table 2. *Effect of different numbers of Protozoa in subcultures on the rates of reproduction for the first 48 hr.*

| Inoculum c.c. | No. of bacteria per c.c. (millions) | No. of *Colpoda* per c.c. | Ratio | No. of bacteria per c.c. after 48 hr. (millions) | No. of *Colpoda* per c.c. after 48 hr. | Ratio | Reproductive rate of 48 hr. |
|---|---|---|---|---|---|---|---|
| 0·5 | 1200 | 4,000 | 300,000 | 960 | 39,200 | 24,489 | 3·29 |
| 1·0 | 1200 | 5,600 | 214,285 | 720 | 64,800 | 11,111 | 3·52 |
| 2·0 | 1660 | 12,400 | 133,871 | 860 | 31,200 | 27,589 | 1·33 |

In mass cultures the rate of reproduction is lower when the number of individuals in a subculture is increased considerably. Table 2 shows the reproductive rates in *Colpoda* for the first 48 hr. The amount of fluid, in which the subcultures were made, was the same in all

cases. In all the tables the bacterial numbers are expressed as a ratio, obtained by dividing the number of bacteria by that of the Protozoa.

Table 3 shows the reproductive rates in *Colpoda* for the first 24 hr. with bacterial food supply of 07, 1734, R and S 21. In all these experiments the amount of the inoculum and the culture fluid was nearly the same, and the cultures, from which the subcultures were made, were all of the same age (48 hr.). With bacterial food supply of 07 and 1734 there is very little difference in the reproductive rates of *Colpoda*. With species R the rate is lower than with 07 and 1734, although the number of Protozoa in the subculture is only 2800/c.c. compared with the numbers in 07 and 1734 (5600 individuals/c.c.). With species S 21 as food supply, the rate of reproduction in *Colpoda* for the first 24 hr. is 0·90. Here the initial number of individuals inoculated is greater than in the case of 07 and 1734, but there is a very marked difference in the reproductive rate in S 21 compared with 07 and 1734. It has been shown that amoebae prefer species S 21 to 07 and 1734, and species R is non-edible (Singh, 1941).

TABLE 3. *Reproductive rates in* Colpoda steinii *for the first 24 hr. with different bacterial food supply. Subcultures were made from 48 hr. old parents*

| Bacteria | No. of bacteria per c.c. (millions) | No. of *Colpoda* per c.c. | Ratio | No. of bacteria per c.c. after 24 hr. (millions) | No. of *Colpoda* per c.c. after 24 hr. | Ratio | Reproductive rate of 24 hr. |
|---|---|---|---|---|---|---|---|
| 07 | 1680 | 5600 | 300,000 | 820 | 58,800 | 13,946 | 3·39 |
| 1734 | 1440 | 5600 | 257,142 | 1000 | 41,200 | 24,271 | 2·87 |
| R | 1660 | 2800 | 578,573 | 1700 | 10,400 | 163,461 | 1·89 |
| S 21 | 1520 | 6400 | 227,500 | 1360 | 12,000 | 113,333 | 0·90 |

In the strain of nodule bacteria (310) *Colpoda* does not reproduce, and even if it divides the reproduction is very slow. Large numbers of species 310 were inoculated into 10 c.c. of soil extract. Later the Protozoa from a 48 hr. old culture, maintained with species S 21, were inoculated. There was very little food in the culture from which the inoculum was made. The initial number of Protozoa in the subculture was 1200/c.c.; after 24 hr. the number fell to 600/c.c., and after another 24 hr. the number of Protozoa was decreased so much that it was not possible to count them by the method used. There were many resistant cysts present in the culture and the individuals were very small. On the third day the number of Protozoa increased to 6000/c.c., and some of the individuals were big; some dividing cysts were also present. After another 2 days the number fell to 1200/c.c., although species 310 was present in quantity. The individuals were small, and resistant cysts were numerous. Such a result has been obtained several times when a subculture was made in which the food supply was species 310.

The experiment suggests that the Protozoa eat species 310 little if at all. The number of *Colpoda* increases with the increase of the contaminating species S 21, and in its absence the Protozoa become progressively smaller and finally encyst.

### (*b*) *The effect of bacterial and protozoal products on the rates of reproduction*

*Exp.* 1. Into a small flask containing soil extract, species S 21 and *Colpoda* were inoculated, and were incubated at a temperature of 19–20° C. After a few days, when the *Colpoda* had eaten nearly all the bacteria, more of the same species were supplied and the flask was incubated for a period of

7–10 days. Then the soil extract was filtered and the filtrate was divided into two small flasks each containing 9·5 c.c. The soil extract of one flask was heated to 70–80° C. for 1 hr. and then cooled. In a third flask 9·5 c.c. of sterile soil extract was taken as control. The three flasks containing the same amount of liquid were first inoculated with S 21 and then with approximately equal numbers of *Colpoda* from a 48 hr. old culture, which had been growing on the same bacterial food supply. The rates of reproduction in the three cases are given in Table 4.

TABLE 4. *Reproductive rates of the first* 48 *hr. in* Colpoda steinii *in the presence and absence of the bacterial and protozoal metabolic products*

| Bacteria | No. of bacteria per c.c. (millions) | No. of *Colpoda* per c.c. | Ratio | No. of bacteria per c.c. after 48 hr. (millions) | No. of *Colpoda* per c.c. after 48 hr. | Ratio | Reproductive rate of 48 hr. |
|---|---|---|---|---|---|---|---|
| S 21 (Control) | 1360 | 1200 | 1,333,333 | 660 | 47,600 | 13,865 | 5·30 |
| S 21 (Filtrate) | 1180 | 1000 | 1,180,000 | 820 | 13,600 | 59,944 | 3·76 |
| S 21 (Filtrate heated for 1 hr. at 70–80° C.) | 1000 | 800 | 1,250,000 | 680 | 37,600 | 18,085 | 5·55 |

It is clear that the rates of reproduction in the control and in the filtrate, which was heated to 70–80° C. for 1 hr., are the same. The rate of reproduction in the filtrate, which was not heated, is slightly lower than in the other two cases. It would seem that either the bacteria or the Protozoa, or both, produce some toxic substance which is thermolabile at a temperature of 70–80° C.

*Exp.* 2. The bacteria (310) were inoculated into sterile soil extract and the liquid was incubated at 25° C. for 10 days. The soil extract was filtered at the end of this period. The experiment was arranged in the same way as the previous one. The results obtained are shown in Table 5.

TABLE 5. *Effect of the presence of the filtrate of nodule bacteria* (310) *and its absence on the rate of reproduction in* Colpoda steinii

| Bacteria | No. of bacteria per c.c. (millions) | No. of *Colpoda* per c.c. | Ratio | No. of bacteria per c.c. after 24 hr. (millions) | No. of *Colpoda* per c.c. after 24 hr. | Ratio | Reproductive rate of the first 24 hr. |
|---|---|---|---|---|---|---|---|
| S 21 (Control) | 1720 | 4000 | 430,000 | 1380 | 22,400 | 61,607 | 2·48 |
| S 21 (Filtrate of nodule bacteria) | 1740 | 3600 | 483,333 | 1540 | 12,400 | 124,193 | 1·78 |
| S 21 (Filtrate of nodule bacteria heated for 1 hr. at 70–80° C.) | 1900 | 3600 | 527,777 | 1540 | 19,200 | 80,308 | 2·41 |

The product of the nodule bacteria, which is of the nature of a growth-promoting substance for plants, has no effect on the rate of reproduction in *Colpoda*. The unheated filtrate of the nodule bacteria seems to depress very slightly the reproductive rate of *Colpoda* during the first 24 hr.

### (*c*) *The rate of reproduction in isolated individuals*

Single individuals washed several times in sterile soil extract were transferred to two drops of soil extract in hollow-ground slides and supplied with approximately the same amount of species S 21 as food supply. The slides were put into moist chambers. Similarly two and four individuals were

isolated, washed and were put into two drops of soil extract and supplied with approximately the same amount of species S 21 as food supply. The chambers were incubated at a temperature of 19–20° C. for 24 hr., and at the end of this period the animals were killed by lugol solution. The number of individuals present was counted and their rates of reproduction for the first 24 hr. calculated. All the animals that were isolated came from cultures of the same age, maintained on species S 21 as food supply.

The average reproductive rates show no evidence of allelocatalysis, and the rates of reproduction are practically the same whether one, two or four individuals are isolated in the same amount of culture medium containing nearly the same amount of food supply (Table 6).

TABLE 6. *Rate of reproduction during the first 24 hr. in one, two and four individuals*

| | 1 animal | 2 animals | 4 animals |
|---|---|---|---|
| Average reproductive rate during the first 24 hr. | 3·71 | 3·67 | 3·61 |
| Number of cases | 8 | 8 | 8 |

As already mentioned, in mass cultures, the rates of reproduction fall when the amount of inoculum is increased. The results obtained in mass cultures are more reliable than when one, two or four individuals are isolated. In isolating and washing individuals it is possible that injuries may be caused to the cells, which may not only stop their reproduction but may induce death after some time. The death of weak and unfit individuals in mass cultures by the shock of being transferred to a fresh medium or by some other cause would not affect the results to such an extent as it would do in the case of one, two or four isolated individuals.

### (*d*) *Encystation*

Of the two types of cysts in *Colpoda* the "reproductive cyst" is several times larger than the resistant or "dauer cyst". From each reproductive cyst two, four or eight individuals emerge by a rupture in the wall. I have generally observed four individuals emerging from a single reproductive cyst. In *Colpoda*, crowding, hydrogen ion concentration, excretion products, and lack of food have been claimed by various workers to be the inducers of encystment (Barker & Taylor, 1931; Taylor & Strickland, 1938). The present work mainly deals with the food factor and the excretion products, though other factors have also been taken into account.

In experiments to test the nutritive values of different species of bacteria it was noted, in every case, that the resistant cysts are formed when the bacterial food supply was almost or completely exhausted. The resistant cysts are never formed when the individuals are large. The time taken by the individuals to encyst depends upon the amount of undigested food present in the body of the animals.

An experiment was set up in which food was added over a period of 9 days, whenever there was scarcity of food in the culture. Into 10 c.c. of soil extract large numbers of bacteria S 21 were inoculated, and then the Protozoa from a 72 hr. old culture. After the first maximal number of Protozoa (81,600/c.c.) was reached the individuals began to encyst, and at this stage no reproductive cysts were present, and all the individuals were very much smaller than those found in a well-fed culture. Plenty of species S 21 was supplied when the number of Protozoa was 51,200/c.c., at which stage only very few resistant cysts were present in the culture. After the addition of food the number of Protozoa per c.c. decreased for a time, this being due to the death of a large number of individuals and not to cyst formation.

A few hours after the supply of food the size of the individuals began to increase and a few reproductive cysts were present in the culture. Later all the individuals were large, and numerous reproductive cysts were present. The number of Protozoa rose to 312,000/c.c. and there were practically no small individuals and no resistant cysts in the culture medium. As the food supply was nearly finished, several loopfuls of bacteria were added. This led to a sudden fall in the protozoal number, shown clearly by the subsequent counts. Although the number of Protozoa fell from 312,000/c.c. to 31,600/c.c. no resistant cysts were formed in the culture. This was due to death of individuals, which in aged or nearly starved cultures of 5–10 days old has been repeatedly observed after heavy supply of bacterial food. If counting had been continued there would have been further increase in the protozoal number, as was observed before, but as little soil extract remained the experiment was discontinued.

Sometimes practically all the Protozoa die in old cultures when several loopfuls of bacterial food are added, and when the individuals are small and no reproductive cysts are present. This phenomenon has been observed in *Colpoda dudenaria* by Taylor & Strickland (1938), who state: "The lethal effect of a dense concentration of these bacteria on *Colpoda dudenaria* is due primarily to the lack of oxygen, and that under the conditions of this experiment the metabolites neither prevent division nor induce encystment."

In previous experiments, it was shown that Protozoa and bacteria produce a thermolabile substance which has a slight effect on the rate of reproduction. It may be possible that the accumulation of large amounts of toxic products may have caused the death of numerous weak and unfit individuals which had become very small owing to the lack of food in the previous experiment. It has been observed that in an old and well-fed culture the death-rate of the individuals after heavy inoculation is not so great as in starved cultures. As pointed out by Taylor & Strickland (1938) it may be possible that the lack of oxygen may also be responsible for the death of the individuals. I have never observed the formation of resistant cysts in the presence of toxic substances produced by bacteria and Protozoa.

In the presence of unfavourable bacterial food permanent cysts may be formed. *Colpoda* is unable to grow when nodule bacterium 310 is supplied as food and when starved individuals, which have become small, are inoculated into soil extract containing bacterial species 310 resistant cysts are formed within a few hours. If well-fed animals are inoculated, they become smaller and smaller and finally encyst. Some of these Protozoa excyst but they again encyst, on account of the lack of favourable food.

In *Colpoda* temperatures up to 25° C. have no effect in inducing cyst formation in the presence of food. No relationship between the age of the culture and cyst formation could be noted, and it does not seem, in the present case, that physiological periodicity, crowding, the age of the culture and the abundance of food are the causes of cyst formation. When a culture containing abundant food is dried the individuals die instead of forming resistant cysts. It was shown by Taylor & Strickland (1938) that growth and encystment occurs in *Colpoda dudenaria* as readily at a *p*H value of 8·2 as that of *p*H 6·0.

### (*e*) *Excystation*

In liquid medium (soil extract, hay infusion, etc.) freshly formed resistant cysts excyst both in the presence and absence of food supply. The mechanism by which a single individual emerges from the resistant cyst is the same as described by Goodey (1913). After their

emergence, when there is sufficient food supply, the individuals increase in size and form reproductive cysts: in the absence of food supply the individuals encyst again within a few hours. A large proportion of resistant cysts do not excyst even if there is sufficient amount of food supply. Hollow-ground slides containing resistant cysts were incubated both at 20 and 25° C.: some were supplied with bacterial food and the others were not. In such experiments excystation occurred both in the presence and absence of food only in the case of recently formed cysts. More cysts excysted when they were kept at 25° C. than when they were kept at 20° C.

The resistant cysts, which had been formed in hollow-ground slides when the food supply was finished, were dried by allowing the soil extract to evaporate at a temperature of 25° C. They were then kept in these hollow-ground slides for more than 3 months at a temperature of 25° C. At the end of this period the cysts were moistened with a few drops of soil extract and in addition some were supplied with bacteria S 21. The cysts were incubated at 25° C. in a moist chamber. In 12–24 hr. many cysts containing species S 21 excysted and later the individuals increased in size and reproduction took place by the formation of reproductive cysts. In the presence of soil extract excystation did not occur up to 7 days. At the end of this period, when many contaminating bacteria had developed, some of the resting cysts excysted. It has been reported by Barker & Taylor (1933), Thimann & Barker (1934) and Taylor & Strickland (1935) that extracts of vegetable or animal tissues induce excystment of *Colpoda*. This experiment shows that the bacteria produce some substance which induces some of the dried cysts, of more than 3 months old, to excyst. It may be possible that many of these resistant cysts after a long period, in the dried condition, were dead, and so were incapable of excystation. Goodey (1913) found that if *Colpoda* cysts were kept in dry condition for a few weeks their power of excystation was rapidly diminished, but in the recently formed cysts excystation was most rapid. The excystation took place in distilled water, tap water, hay infusion and in soil extract, but was inhibited in an acid medium. Rhumbler (1888) could not keep dried cysts of *Colpoda* more than 3 weeks. Many resistant cysts, whether they are freshly formed or dried for a long time, do not excyst under the conditions of the present experiment. When the cysts are left in the dried condition in hollow-ground slides for more than a year, they are incapable of excystation and all of them die.

## Discussion

The literature on allelocatalysis and the effect of different bacteria on the growth and reproductive rates of Protozoa has been summarized in two recent reviews by Luck *et al.* (1931) and Hammond (1938). It is enough to point out that there is no allelocatalysis in *Colpoda*, and that different types of bacteria influence the growth and the reproductive rates in Protozoa.

When bacterized hay infusion is used as food for the ciliates, they may encyst when the favourable bacterial food supply is finished, even if the amount of the unfavourable food increases to a large extent. It is possible that some of the results obtained on encystation in ciliates, in the presence of food, may be due to this reason. Such a result has been obtained in *Colpoda* in the presence of a strain of nodule bacteria. If one is studying the food factor in connexion with encystation in ciliates and other Protozoa it is of the utmost importance to work under sterile conditions and to use only one type of favourable bacteria as food.

Food is not the only factor which induces cyst formation in Protozoa. The writer has observed that in a liquid medium, soil amoebae encyst even in the presence of favourable food supply. In this case temperature, metabolic products, etc., seem also to influence the induction of cyst formation. In different ciliates other factors, apart from the food, may influence cyst formation, but many of the controversial results which have been obtained are probably due to the fact that the experiments were not carried out under sterile conditions, and that a favourable bacterial food supply was not used.

## Summary

1. The rate of reproduction in *Colpoda* varies considerably with the bacterial food supply, the age of the culture, the size of inoculum, and the metabolic products.

2. The metabolic products of bacteria and Protozoa have a slight retarding effect on the rate of reproduction. There is no evidence of allelocatalysis in *Colpoda* either in isolated individuals or in mass cultures. The evidence obtained in mass cultures shows that the rate of reproduction in a subculture containing a smaller number of Protozoa is better than when the number is considerably increased.

3. The resistant or "dauer" cysts are formed only when there is practically no bacterial food present in the culture solution or in the presence of unfavourable food supply, e.g. strain of nodule bacteria 310. The metabolic products of bacteria and Protozoa and certain other factors have no influence on the formation of resistant cysts in *Colpoda*.

4. Encystation takes place even in dried cysts of more than 3 months in the presence of bacteria. No excystation takes place in such dried cysts when they are moistened with only soil extract without the presence of bacteria. Thus it seems that bacteria produce some substance which induces excystation in the case of *Colpoda*.

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# NOTE

## SEASONAL OCCURRENCE OF THE TAKE-ALL DISEASE OF WHEAT

By W. BUDDIN,
*University of Reading*
AND S. D. GARRETT,
*Rothamsted Experimental Station, Harpenden*

DURING 1940, few cases of the take-all or whiteheads disease (*Ophiobolus graminis*) were found in wheat crops, even on the light-textured chalk soils on which they are usually to be expected. The apparent freedom of the crops from this disease is probably to be attributed to the dryness of the season; take-all was also somewhat scarcer than usual in 1938, in which year a spring and early summer drought was experienced. An association between epidemics of take-all and wet seasons was reported by Russell (1930) in Canada and by Garrett (1934) in South Australia. The suggestion that mycelial advance of *Ophiobolus* along the roots is stopped by dryness of the soil is supported by our observations on numerous samples taken from wheat crops during 1940. Infection by *Ophiobolus* was frequently to be found on the seminal roots and on the lower parts of the crown roots, but it had failed to advance to the crown.

These observations imply, therefore, that many wheat crops which looked perfectly healthy and showed no whiteheads at harvest were actually carrying *Ophiobolus* infection on their roots. Under present conditions, farmers may decide to risk a second crop of wheat after a first crop free from whiteheads; the danger of such a practice may be illustrated by an experience of our own. In 1937, two plots, 22 × 16 ft., of White Victor winter wheat in cages in use by one of us (W. B.) were ruined by take-all; almost every plant in both cages was a whitehead, and showed characteristic stem-blackening by *Ophiobolus*. The crop was cut and the stubble dug under in both cages on 10 and 11 Aug.; White Victor wheat was sown on the following 28 Feb. This spring wheat crop appeared almost completely healthy at harvest; a few *Ophiobolus* whiteheads were present in either cage. Failure of the disease to develop might be ascribed to (1) dying out of the fungus from infected root and stubble residues during the 6½ months interval between 11 Aug. and 28 Feb. of the following year, (2) so firm a settling of the soil in the two cages as to check subterranean spread of *Ophiobolus* along the roots of the spring wheat crop or (3) the dry weather experienced during spring and early summer of 1938. Roots of some of the apparently healthy plants from one of these cages were examined; some *Ophiobolus* root infection was found, to which insufficient importance was attached at the time. The stubble of this spring wheat crop was dug-under on 8 Sept., and Wilhelmina winter wheat planted on 11 Oct. As the 1939 crop matured, whiteheads appeared throughout both cages, and by harvest the crop was found to be a total failure; plate mycelium of *Ophiobolus* was found at the base of every whitehead plant examined.

These observations show, therefore, that a wheat crop apparently almost free from the take-all disease at harvest may in reality be carrying sufficient infection on its roots to ruin a following winter wheat crop if soil and seasonal conditions permit.

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# REVIEWS

*The Virus: Life's Enemy.* By KENNETH M. SMITH. Pp. viii + 176. Cambridge: University Press. 1940. 7*s.* 6*d.*

A well-balanced general account of human, animal, and plant viruses and virus diseases, written for the scientific worker in other fields and for the educated layman; illustrated by a frontispiece, 8 plates and 1 text-figure. The writing is simple, clear, and descriptive, and the book, in spite of its somewhat catch-penny subtitle, is one to be cordially recommended. I have found it particularly useful in arousing the interest of agricultural and horticultural students in the wider aspects of these problems.

W. B. BRIERLEY

*A Survey of Insecticidal Materials of Vegetable Origin.* Edited by H. J. HOLMAN. Pp. viii + 155. London: Imperial Institute. 1940. 3*s.* 6*d.*

The Consultative Committee of the Imperial Institute which deals with the above subject appointed a subcommittee which sent out questionnaires to appropriate Government Departments overseas, and also consulted the Governments of Burma and the Anglo-Egyptian Sudan. The material so gathered supplemented by already published work is the basis of this monograph. Part I (pp. 27) deals with alkaloid-containing materials: nicotine and nicotine products from *Nicotiana tabacum* and *N. rustica*, anabasine from *Anabasis aphylla*, hellebore from *Veratrum album* and *V. viride*, and other alkaloids having insecticidal action from a variety of plants. Part II (pp. 65) deals with plants containing rotenone and allied compounds: species of *Derris*, *Lonchocarpus*, *Tephrosia*, *Mundulea*, and *Millettia*. Part III (pp. 37) deals with pyrethrum, obtained from *Chrysanthemum cinerariifolium* and to a much less extent from *C. coccineum*. Part IV (pp. 6) deals with Jamaica quassia obtained from *Picroena excelsa* and Surinam quassia from *Quassia amara*. Part V (pp. 2) deals with certain plant oils. There is an informative Introduction, an excellent Bibliography, and an Appendix giving chemical structural formulae of certain active agents. The survey contains an astonishing amount of information on the sources, botany, and cultivation of the plants, the chemistry of the compounds and their physiological and insecticidal action, and the commercial production and world trade of the products. Mr Holman has performed his task well and in every way the monograph is a creditable and most useful publication.

W. B. BRIERLEY

*The Breeding of Herbage Plants in Scandinavia and Finland.* Pp. 124. Imperial Agricultural Bureaux Joint Publication, No. 3. Aberystwyth: Imperial Bureau of Pasture and Forage Crops; Cambridge: Imperial Bureau of Plant Breeding and Genetics. 1940. 4*s.* 0*d.*

Seven useful practical papers by Nilsson-Leissner, Åkerberg, and Torssell of Sweden, Frandsen of Denmark, Wexelsen of Norway, and Pohjakallio of Finland, on the cytogenetics and breeding of red and white clovers, alsike, lucerne, and herbage species of *Agrostis*, *Alopecurus*, *Arrhenatherum*, *Bromus*, *Dactylis*, *Festuca*, *Lolium*, *Phleum*, and *Poa*: most attention is given to red clover and timothy. In red clover there seems promise of strain resistance to *Tylenchus devastatrix*, *Sclerotinia trifoliorum*, *Erysiphe polygoni*, and *Peronospora trifoliorum*; in timothy to *Sclerotinia borealis* and *Puccinia phlei-pratensis*; in alsike to *Sclerotinia trifoliorum*; and in perennial ryegrass to *Puccinia coronata* f. *lolii*.

W. B. BRIERLEY

*Vegetative Propagation of Tropical and Sub-Tropical Plantation Crops.* By G. ST CLAIR FEILDEN and R. J. GARNER. Pp. 99. Technical Communication, No. 13. East Malling: Imperial Bureau of Horticulture and Plantation Crops. 1940. 3*s.* 6*d.*

A useful companion bulletin to that on Fruit Crops published by the same authors in 1936. The first 16 pages contain a concise, practical and well-illustrated account of methods of vegetative propagation, and the remaining pages deal in detail with the application of these methods to fifty-five plantation crops arranged alphabetically by genera. Bibliographies are appended to the individual crops. The compilation of this bulletin represents an enormous amount of work but, of its kind, it could hardly be bettered.

W. B. BRIERLEY

*The New Systematics*. Edited by JULIAN HUXLEY. Pp. viii + 583. Oxford: Clarendon Press. 1940. 21*s*. 0*d*.

This volume, sponsored by the Association for the Study of Systematics in relation to General Biology, contains an introductory survey by Huxley, followed by twenty-one essays on new, not so new, and rather elderly systematics, by a variety of botanists and zoologists, mostly British, and ranging from crude modernity to period pieces. The essays vary in length and type from Calman's chatty five pages on "A museum zoologist's view of taxonomy" or Salisbury's rather elegant 12-page essay on "Ecological aspects of plant taxonomy" to Timofeeff-Ressovsky's 64 slightly ponderous pages with 197 bibliographic references on "Mutation and geographical variation" or Muller's 74 rather turgid pages with 205 references on "Bearing of the *Drosophila* work on systematics". The lengths of the essays in no way relate to the importance of the topics, and no effort seems to have been made to introduce any sense of balance among authors or subjects, or to plan out and cover adequately the whole field: they are just diverse essays by divers authors. Still, as the editor admits in his foreword, "The committee is fully conscious of the somewhat presumptuous sound of the title it chose for the book". From every point of view, however, the book is infinitely worth the labour of its production. Botanists and zoologists of every kind must try to clear their minds on the terms and concepts of daily usage, and of all biological problems those of systematics are most fundamental. But all too long have the museum and herbarium taxonomists been the arbiters of our usage, and this symposium would have been even better had the professional systematist been excluded from participation in its authorship. The more fundamental and revolutionary changes in systematic concepts to-day derive not at all from taxonomists who, almost to a man, are conservatively minded, but from the experimentalists, from biogeographers, ecologists, geneticists, plant pathologists and other workers in one or another province of applied biology. I know of few recent books of more general interest and value as background reading or more stimulative of thought than this volume, although I also know of few symposia in which there is so little general agreement between authors on any one of the major concepts involved in their writings. The authors contributing to the volume are as follows: H. H. Allan, W. J. Arkell, W. T. Calman, M. B. Crane, C. D. Darlington, G. R. de Beer, C. Diver, E. B. Ford, J. S. L. Gilmour, L. Hogben, J. A. Moy-Thomas, H. J. Muller, J. Ramsbottom, E. J. Salisbury, J. Smart, T. A. Sprague, W. H. Thorpe, N. W. Timofeeff-Ressovsky, W. B. Turrill, N. I. Vavilov, E. B. Worthington and Sewall Wright.

W. B. BRIERLEY

*Plant Microtechnique*. By D. A. JOHANSEN. Pp. xii + 523. London: McGraw-Hill Publishing Co., Ltd. 1940. 30*s*. 0*d*.

Since the early days of Zimmermann and of Bolles Lee, microtechnicians have served their subject well, and at frequent intervals new editions and new works have appeared describing and co-ordinating the progress of the subject: the present volume is no mean addition to this literature. The familiar "Chamberlain" last appeared in 1932, since when there have been striking advances in methods and procedures and much sifting of experience. For 16 years or so Prof. Johansen has worked intensively on these problems covering the entire range of the plant kingdom, and in this book he has not only made a critical survey in the light of his experience but has included much of his own unpublished work. In the first half of the book he describes the apparatus, reagents, dyes, etc., and the general methods and procedures employed by botanical technicians. In the second half he works through the plant phyla in phylogenetic order, giving detailed suggestions or directions for the treatment of specific groups in each phylum. To many workers Part II may seem considerably unbalanced, since of the fifteen chapters the algae receive seven, whilst the bacteria, myxomycetes, fungi, bryophytes, ferns, cycads, conifers, and flowering plants receive only one each. There is a bibliography of about 250 references cited in the text and an adequate index.

Although the book contains an enormous number of data, methods, etc., it does not set out to be an encyclopaedia of botanical microtechnique. The author has excluded all dyes, techniques, etc., which have not proved their value and, with few exceptions, every procedure cited has been tested by the author or his students. Prof. Johansen, however, writes from the sunny, peaceful State of California and, as he points out on p. 174, "Statements in the literature to the effect that such-and-such a fluid 'gives the best results', without specifying under what conditions such results were obtained, are meaningless. Conditions are not the same everywhere at all times. In England, for example, La Cour's fluids are popular and apparently afford excellent results, but on the Pacific coast the fixation is

atrocious, and staining is most difficult. Climatic conditions apparently have a definite bearing on fixation results and certainly have one on the subsequent staining." Usually this truism is entirely overlooked by nine out of ten workers, and it probably explains what may seem sins of omission or of commission, or unusual or unexpected particularities in the book. By and large, however, Prof. Johansen has produced the best volume to date on his subject and botanists the world over stand in his debt.

W. B. BRIERLEY

*A Manual of Aquatic Plants.* By N. C. FASSETT. Pp. vii + 382. London: McGraw-Hill Publishing Co., Ltd. 1940. 26*s.* 0*d.*

The author defines an aquatic plant as one "that may under normal conditions, germinate and grow with its base in the water and is large enough to be seen with the naked eye"; the plants coming within this category range from filamentous algae upwards; and the region covered by the author is from Minnesota to Missouri and eastward to the Gulf of St Lawrence and Virginia, U.S.A. The book consists of an enormous number of excellent line drawings of whole or dissected plants accompanied by systematic keys which are essentially a set of directions for looking at the pictures. There is an interesting appendix on the use of aquatic plants by mammals, birds and fish, together with useful bibliographies, and the book concludes with a glossary and index. Many of the drawings and keys, and much of the information in the book will be useful to non-American workers. W. B. BRIERLEY

*The Orientation of Animals: Kineses, Taxes and Compass Reactions.* By G. FRAENKEL and D. L. GUNN. Pp. vii + 352 Oxford: Clarendon Press. 1940. 21*s.* 0*d.*

In its undergraduate days my generation was thrilled by Jennings' *Behaviour of the Lower Organisms.* Then in 1918 Jacques Loeb reorientated our views and again keyed our interest to a high pitch in his *Forced Movements, Tropisms and Animal Conduct.* Most of us entirely overlooked Kuhn's *Orientierung der Tiere in Raum,* published in 1919, and his later papers did not come our way, so that we remained biased by Loeb's views which only became slowly modified by the later work of A. S. Russell, Lloyd Morgan, and students of comparative psychology. Apart from a few specialists Kuhn's work, which is of fundamental importance, has been quite inadequately recognized in English-speaking countries. The present authors have remedied this situation: in Part I of their book Dr Gunn has, broadly speaking, reorganized Kuhn's classification, adding certain of von Buddenbrock's categories, whilst in Part II Dr Fraenkel has reviewed the literature in the light of this revised classification. At first sight this book may seem of little applied interest, but if one thinks of pest and disease relationships in terms of orientation of parasites to environmental factors with conditioning by internal factors, then the importance of this fundamental study becomes clear. The book seems to me a major contribution to the subject of animal behaviour. W. B. BRIERLEY

*Mechanisms of Biological Oxidations.* By D. E. GREEN. Pp. 181. Cambridge: University Press. 1940. 12*s.* 0*d.*

The main concern of this book is the mechanisms of cellular oxidation, the oxidation enzymes being classified according to the chemical nature of their prosthetic group. Following an introductory chapter on the general properties of oxidation enzymes, five chapters deal with enzymes with known prosthetic groups (containing copper, iron-porphyrin, zinc, flavin, nicotinicamide, thiamine, glutathione), whilst the last three chapters deal with enzymes whose prosthetic groups, if any, are still unknown (cytochrome-reducing dehydrogenases, unclassified oxidation enzymes, and oxidations in organized systems). Each chapter is terminated by a good bibliography. For those of us who have no first-hand acquaintance with this difficult and rather obscure field of biochemical research the book does not make easy reading, but the effort is worth while, since these mechanisms underlie so many of the functional activities and relations whose superficial expressions in organisms are so familiar to us in our daily work. W. B. BRIERLEY

*Statistical Calculation for Beginners.* By E. G. CHAMBERS, M.A. Pp. viii + 110. Cambridge: University Press. 1940. 7*s.* 6*d.* net.

This terse book contains lucid directions for calculating and interpreting all the elementary statistics. It assumes no knowledge of mathematics beyond arithmetic and ability to substitute in simple algebraic formulae. There are copious worked examples and exercises with answers. Its scope extends to tests for normality of distribution and linearity of regression; but, though the $z$-test is dealt with, factorial analysis is not mentioned. It will thus not fully satisfy the needs of agricultural experimenters. There is an excellent little section on the "use and abuse of statistics". One would like to see as much—or more—in all elementary text-books of this kind. P. WHITE

*Embryos and Ancestors.* By G. R. DE BEER. Pp. x + 108. Oxford: Clarendon Press. 1940. 7*s.* 6*d.*

This book is an up-to-date and enlarged edition of the author's *Embryology and Evolution* published ten years ago and out of print for some time. The author presses his rejection of the still widely held recapitulation theory of Haeckel, briefly but cogently synthesizes the existing state of knowledge in the fields of development, evolution and heredity, and then produces a consistent and co-ordinating formula based on repetition due to the transmission of internal factors from ancestor to descendant. An interesting and important little book. W. B. BRIERLEY

*Fundamentals of Biology.* By A. W. HAUPT. Third edition. Pp. xii + 443. London: McGraw-Hill Publishing Co., Ltd. 1940. 20*s.* 0*d.*

Beginning with protoplasm and the cell and ending with the evolution of man, this is a good general introduction to biology as a cultural rather than a technical study. In this edition minor changes have been made throughout, and there is a fuller treatment of animal co-ordination, nutrition, and disease. W. B. BRIERLEY

*Principles of Animal Biology.* By LANCELOT HOGBEN. Second edition. Pp. 415. London: George Allen and Unwin, Ltd. 1940. 7*s.* 6*d.*

Originally published in 1930 and out of print for some years, the text has been revised and partly rewritten, and the illustrations have been redrawn by Mr J. F. Horrabin. The book is a good general introduction to the subject. W. B. BRIERLEY

*Man on his Nature.* By Sir CHARLES SHERRINGTON. The Gifford Lectures, Edinburgh, 1937–8. Pp. 413. Cambridge: at the University Press. 1940. 21*s.* 0*d.*

This book of Gifford Lectures is adapted, in method of approach and style of presentation, to the conditions of its origin. It is none the less highly to be commended to applied biologists and, indeed, to all specialists whose enforced concentration on their own focal problems tends to limit their appreciation of the advance of science as a whole. The author himself has surmounted any such tendency. His survey of the nature of man offers a bird's-eye view of the findings and trend of thought of natural science (i.e. excluding psychology) in many of its major aspects, including some of the results of his own life-work on the nervous system. The distinctive features of modern thought are brought into relief by comparison with views held by Jean Fernel, philosopher-physician of the sixteenth century. MARJORIE BRIERLEY

*The Advance of the Fungi.* By E. C. LARGE. Pp. 488. London: Jonathan Cape. 1940. 18*s.* 0*d.*

I had thought I probably knew everyone in this country who might be capable of writing a major work on the fungi and I was, therefore, a little intrigued when I received this volume. In these days, especially, one does not lightly undertake to read nearly 500 closely printed demy octavo pages by an author whom one has no reason to suspect of possessing any serious knowledge of his subject. That the author acknowledges the help of Pethybridge and Bisby, both of whom read the whole book in typescript, at least guaranteed the book's freedom from scientific error but still did not prepare me for

what I found. Let me say at once that not only does Mr Large possess a detailed technical knowledge and sympathetic understanding of his subject but he has written a perfectly fascinating book which, in its field, is unique.

Stated prosaically, the volume contains 31 chapters giving an account of various epidemics (or, as Whetzel would say, 'epiphytotics') of fungal and other plant diseases and of the development of preventive measures, and closes with a 26-page classified bibliography: it is illustrated by 6 plates and 58 text-figures. In writing a notice of such a book one could retain the narrower professional attitude, treating the work purely as a history of phytopathology, a kind of super Whetzel or Braun and Morstatt. In so analysing it one would find it somewhat partial and selective, often redundant and at times even confused, although one would admit the author's historical accuracy, and admire his pertinacity in delving into old records. One would emphasize the author's wide reading, his understanding of disease-causing agencies and of disease relationships, his fair but not always balanced consideration of the many facets of his subject, and the wealth of mycological and of phytopathological data embodied in his book. One would, perhaps, especially draw attention to the splendid way in which the author portrays the development of the idea of the prevention and control of plant disease, his obvious first-hand knowledge of modern work in this field, his insight into the wider agricultural and economic relationships of these problems, and his clear vision of present and future trends. And in proceeding chapter by chapter through the book one would remark upon its many helpful ideas and fruitful suggestions, one would find many things to praise but also many sins of omission and of commission. The book is literally studded with points for discussion and every plant pathologist reading it will surely wish to rewrite greater or lesser portions. And all of this would be eminently correct and, at the same time, it would I think almost wholly miss the value and purpose of Mr Large's book.

True, the author's approach to his subject is historical and his attitude is scientific, but his writing is pictorial and dramatic, and whilst he imparts valuable and interesting knowledge this is entirely secondary to his main purpose which is gradually to persuade his reader to an attitude of mind and to create in his reader a mental picture. Facts there are a plenty, but for Mr Large they have no great intrinsic value, they are merely a framework on which to arrange ideas. He is not concerned to stuff his readers' minds with mere data, nowadays one keeps one's memory on one's bookshelves, but he is very much concerned that the reader shall gradually visualize crop plants and their diseases not only as things of intrinsic intellectual interest but as things of vital social and economic importance, as key factors in the development of human civilization. Blighted potatoes and rusted wheat are not merely interesting specimens for laboratory study, they are primary limiting factors in the world's agricultural economy, in the production of the daily food of hundreds of millions of men and women.

Thus, the early chapters of the book are pregnant with the social history of Europe in the mid-nineteenth century. Poland, Germany, Belgium, Holland, France and England, all showed famine faces, and then the 'murrain' struck Ireland. It recognized no boundary between a future Ulster and an Eire, and the potato fields of Catholic and Protestant alike became 'one wide waste of putrefying vegetation'. I often feel great sympathy with those who would de-bunk history. Kings and concubines and the fret of wars, the dementia of dictators, and the changing boundaries of nations may form an obtrusive veneer, but the real stuff of history is bedded in natural phenomena, in the common people, and in the mind of man; an influenza epidemic may kill more people than a world at war, soil erosion or desert encroachment may devastate a continent, changing birth rates may alter the balance of entire populations, new ideas in medicine, in agriculture, or in other branches of knowledge may change the lives of multitudes within a span of years, and new social and economic ideals rumbling volcanically in the minds of common men and women may build a new Jerusalem. It may be true as Blaise Pascal said of Cleopatra's nose that 'had it been shorter, the face of the world had been changed', but far more surely is it true that *Phytophthora infestans*, causing the Irish potato famine, led directly to the repeal of the Corn Laws, 'perhaps the most significant single event in the history of the British Empire', and *P. infestans* causing famine in Europe initiated those tides of humanity which year after year swept from the old world to people the new. This miserable fungus changed the face of the world more vastly and surely than did any queenly nose.

Mr Large can admire the 'unearthly beauty' of Charles Tulasne's 'emotional' illustrations to the *Selecta Fungorum Carpologia*, and reproduces one of its loveliest plates; he can appreciate the scholarly accuracy and dispassionate values of a pure mycological investigation by a De Bary or a Marshall Ward; and in his writing these things live. In his pages the imagery has something of the quality of talking films unrolling the past before one's mind, and in their passage one lives through the decades in which mycology became a science. But far more important during these decades was the growth in peoples' minds of a new conception of the nature of disease, not only in plants but in all living things.

In these chapters the whole development of our present day concepts is traced; a fascinating historical panorama of mycological and phytopathological research in which idea grows out of idea and disease follows disease in scientific procession; and like a golden thread is the gradual crystallization out of scientific values, the lusty values of plant disease and the thinner scholastic values of academic mycology.

But Mr Large's vision goes beyond the immediate phenomena of description and experiment and sees their impact on human life and purpose. For him science is no bloodless abstraction, it is a social function to be visualized in the perspective of human individual and social values, and to be judged in that setting. Plant diseases are not things merely to be studied as an intellectual pursuit or elegance of the mind; they are evil things to be controlled, to be annihilated. And the story of man's attempts to protect and save his crops, from its fumbling, homely beginnings to the modern achievements of the breeder's skill or the triumphs of molecular sprays and dusts is a thrilling chapter in the history of agriculture and of human progress. Yet is this chapter, like many another in the book of man's life on the soil, also a pitiful history of the frustration, of the warping and twisting of the agricultural way of life: it is an indictment of a social and economic system which takes no reck of human happiness and social welfare, and which prostitutes to individual ends and private gain the lives and labours of scientist and peasant alike. If there be one flash of vision, one glimmer of wisdom in this twentieth century of power and knowledge, then there can be no justification for a social and economic system which bids peasants in their poverty to burn their crops, bids starving men to destroy food, bids scientific workers to suppress knowledge or its application to human welfare. These things are sheer insanity stalking the world, a soulless brutalization of human ideals, and a lasting shame to mankind. Agriculture is not a means to private gain, it is the foundation of all human life and all civilized development, it is the skeletal framework of any stable social organization, and the very life blood of a people's sanity.

But, as Mr Large bluntly says, 'The vascular economic system in the organism of society was sodden with profit viruses and rotten from the roots up' and who to-day dare gainsay him? Throughout a century of English history agriculture has been an ugly duckling, only in times of war to be recognized as a swan, but even in war time and even to-day to suffer from that lack of understanding and that myopic vision which has characterized a century of peace-time governments in their relations with this way of life. When will men realize that agriculture is a civilization in itself. Mr Large has thought deeply on the wider economic and state relationships of agriculture, of food production and conservation, and in the particular field of crop protection he has had extensive experience of the commercial exploitation of scientific work. He has formed strong views, views which will out in his writing, and whether the reader agrees with Mr Large or not he cannot dismiss these views lightly.

WILLIAM B. BRIERLEY